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TITLE: THERAPY OF ADULT RESPIRATORY DISTRESS SYNDROME WITH ALPHA-1-ANTIPROTEINASE OR LUNG SURFACTANT

PRINCIPAL INVESTIGATOR: Roger G. Spragg, M.D.

CONTRACTING ORGANIZATION: University of California,
San Diego
P.O. Box 109
LaJolla, California 92093

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Two clinical pilot studies were accomplished. The first focused on the administration of α_1-proteinase inhibitor (α_1-PI) to patients with the adult respiratory distress syndrome and had the goals of determining safety, describing pharmacokinetic parameters, and determining whether intravenous administration of α_1-PI would augment functional antiprotease activity of blood and/or bronchoalveolar lavage fluid. Results indicate: (1) administration of α_1-PI was safe; (2) both initial and steady state clearance of α_1-PI from the central volume of distribution were enhanced; (3) infused α_1-PI retained all of its functional activity; (4) lung lavage levels of α_1-PI did not increase after intravenous infusion of α_1-PI; and (5) the calculated contribution of the infused α_1-PI to those levels was significant. The second pilot study focused on the possible benefits to gas exchange and lung compliance that might accompany administration of exogenous lung surfactant to patients within the first 48 hours of development of ARDS. Results indicate: (1) administration of a single relatively small dose of exogenous surfactant resulted in a transient improvement in gas exchange; (2) in certain cases lavage surfactant inhibitor activity was overcome; (3) physiological parameters of gas exchange and biochemical markers of inflammation indicated no untoward reaction; (4) no alterations in lung compliance or chest radiograph were found following the single 50 mg/kg dose of surfactant administered to patients in this study; and (5) neither immune complexes containing anti-surfactant antibody nor free anti-surfactant antibody were detected in the plasma of patients receiving surfactant.

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FOREWORD

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Roger D Swope 4/15/91

PI Signature Date

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5. INTRODUCTION

Acute lung injury has been known by multiple names including the adult respiratory distress syndrome (ARDS), wet lung, shock lung, capillary leak syndrome, DaNang lung, post-perfusion lung, and congestive atelectasis. Many of these names recall the association between acute pulmonary disease and traumatic battlefield injuries. Thus, as noted by Major L.A. Brewer in 1946, "Experience gained in treating a large number of casualties ... has shown the importance of the 'wet lung' in reference to the morbidity and mortality of patients with wounds of the chest, brain, and abdomen" (1). These authors noted that "in the late stages of shock the capillary permeability is increased in nontraumatic regions of the body so that blood plasma escapes into the tissue spaces. That which escapes into the pulmonary alveoli results in the clinical findings of pulmonary edema." Almost a half century later, interest continues to focus on the consequences of injury to the alveolar-capillary membrane. Ashbaugh *et al.* provided definition of the syndrome in the medical setting (2). Catheterization of the pulmonary artery has helped to distinguish between pulmonary edema associated with elevated pulmonary vascular pressures and that associated with inflammation of the lung. A central focus of current investigation is study of the various biologically active factors that affect permeability of the alveolar-capillary membrane, of the control of expression of those factors, and of their effects on the lung. From such studies come rational proposals for therapeutic interventions, such as the two supported by this contract.

Operational definitions of acute lung injury vary slightly. Many investigators define that injury to be lung edema that occurs over less than 7 days, is present in the absence of pulmonary vascular pressures sufficient to be the primary cause of pulmonary edema, and is diffuse in nature as reflected by pan-lobar infiltrates on the chest radiograph. In the presence of such edema, shunting of pulmonary blood flow and diminished lung compliance are common.

Examination of pulmonary tissue and bronchoalveolar lavage (BAL) fluid from patients with ARDS suggests that a variety of inflammatory mediators may contribute to the lung injury. Lavage fluid contains 1 - 10 mg protein/ml, and these proteins represent all classes of serum proteins (3). Polymorphonuclear leukocytes (PMN), normally less than 2% of cells in BAL, may comprise over 90% of BAL cells; in addition, total cell yield is increased. Analysis of BAL also reveals the presence of a variety of soluble mediators of inflammation.

Histologic examination of pulmonary tissue obtained at autopsy from patients who died after developing ARDS suggests the presence of acute and chronic stages (4). In the first few days of acute lung injury, alveoli are filled with proteinaceous fluid containing red blood cells, neutrophils, macrophages, and cell fragments. The type I epithelial cells are focally destroyed, and endothelial cells may appear swollen. Interstitial edema occurs, and cuffs of edema are seen around bronchioles and vessels. Hyalin membranes composed of fibrin strands and plasma proteins are seen predominately in alveolar ducts. The number of PMNs seen in capillaries is markedly increased; extravasated PMNs are seen in the interstitium and alveoli (5). A more chronic stage of acute lung injury is apparent after 1 to 2 weeks. Cuboidal epithelial cells closely resembling type II cells cover the surfaces of alveoli and alveolar ducts. Proliferation of pericytes and fibroblasts occurs, and plasma cells, histiocytes, and lymphocytes are seen in the interstitium. Intravascular microthrombi are common (6). The acinar architecture of the lung is replaced by thick layers of fibrotic tissue. This fibrosis occurs in a pattern centered on alveolar ducts (7). Total lung collagen is increased two to three-fold after 10 or more days of illness (8). Katzenstein has stressed that the histopathology associated with acute lung injury is non-specific, and is likely to represent

the effects of numerous dissimilar agents (9).

The physiologic changes associated with acute lung injury might be predicted from histologic examination. Compliance of the lung is low. Gas exchange is markedly impaired, and is predominately due to shunt. The resistance of the pulmonary vasculature to blood flow is often increased, and airway resistance is also significantly increased (10).

Estimates of the incidence of ARDS differ greatly among predisposed patient groups. Baumann, *et al.*, noted that ARDS developed in 2% of 4,222 patients, classified as medically acute, when they presented in a hospital emergency room (11). Connors, *et al.*, estimated that ARDS affects 150,000 hospitalized patients yearly in the United States (12). In a retrospective study, an estimated incidence of the syndrome was 50 per 100 patients with sepsis, whereas, in a prospective one-year study conducted in three hospitals, sixty-eight of 993 patients identified at risk subsequently developed ARDS, as did another 20 patients from causes other than those with one or more of eight prospectively identified predisposition (13,14). In the latter study, the incidence rate of the syndrome in patients with one and several predispositions were 5.8 and 24.6 per 100 patients, respectively. In reports spanning the last decade, mortality rates have remained in excess of 50%, in spite of advances in sophisticated therapeutic maneuvers and cardiopulmonary support.

The sequence of pathophysiologic events in the evolution of ARDS is not entirely understood. The syndrome may develop after initial injury to the endothelium via mediators of vascular permeability following non-thoracic insults, or after direct insult to alveolar epithelial cells. Clearly, however, both the endothelium and epithelium become affected in the characteristic pathologic sequence of increased capillary permeability with development of a protein-rich and cell-rich interstitial and alveolar edema (4). A spectrum of morphologic changes including granulocyte entrapment in the microvasculature of the lung, focal collapse of alveoli, hyaline membrane formation, patchy interstitial hemorrhage and fibrosis, and proliferative changes in type II epithelial cells have been noted in lung tissue of patients dying in respiratory distress at various time intervals after oxygen therapy (15).

Protease-antiprotease imbalance in the lungs of patients with acute lung injury:

Recent experimental observations have suggested that although different mediators (e.g., cytokines, complement components, arachidonate metabolites) elicit and sustain neutrophil influx and increased capillary permeability, it may be the release of proteolytic enzymes (elastase, collagenase) and the generation of reactive oxygen species ($O_2^{\cdot}$, H_2O_2, $O_2^{\cdot}$) by the neutrophils that are injurious to the lung. In an *in vitro* system, using cell matrix derived from cultured human umbilical vein endothelial cells, stimulated neutrophils cooperatively used oxygen metabolites and lysosomal elastase to degrade the cell matrix (16). In the presence of high concentrations of alpha-1-proteinase inhibitor (α_1-PI) the ability of stimulated neutrophils to degrade the matrix was impaired. However, when α_1PI was at lower concentration, the degradation potential of the released elastase was enhanced by neutrophil generated oxidants in an H202 dependent process. Since the primary regulator of neutrophil elastase in the lower respiratory tract is α_1PI, it appears that the proteinase-antiproteinase theory of protection of the lower respiratory tract may be, in part, fundamental to some of the pathophysiologic events in ARDS. The proteinase-antiproteinase theory hypothesizes that when pulmonary α_1PI activity is deficient, alveolar structures are unprotected and progressively distorted by proteinase

released by inflammatory cells. Pulmonary α_1PI deficiency may be due to congenital deficiency of α_1-PI or, as recent data suggest, the effect of an acquired functional antiprotease deficiency. The latter may be an consequence of either endogenous or exogenous oxidants (17-20).

Lee, *et al.*, found high levels of the proteolytic enzyme, elastase, in BAL fluids in 12 of 23 patients with ARDS (21). In patients with chronic lung disease and in normal individuals (smokers and non-smokers), infrequent and low levels of elastase activity were noted. The lavage fluids with high elastase activity gave strong precipitin lines when tested against antibody to human neutrophil elastase (HNE), data supporting the hypothesis that the proteolytic activity was of neutrophil origin. Assays for the concentration and the functional activity of α_1PI in BAL fluids suggested that normal concentrations of α_1PI were present in ARDS, but in patients with high elastase activity, there were low levels of functional α_1PI. In this study, no measureable α_1PI: elastase complexes in BAL fluids were noted.

McGuire *et al.*, also demonstrated the presence of HNE in BAL fluids from 17 of 24 patients with ARDS and none of seven normal individuals (22). Variable amounts of HNE were measurable in BAL fluids in 29 of 99 patients with other forms of pulmonary disease (pneumonitis, cancer, chronic obstructive lung disease, etc.). In BAL fluid of seven patients with ARDS, no elastase was noted but each was found to contain inhibitors of proteolytic activity (mainly α_1-PI). In some of the patients with active elastase in BAL fluids, α_1PI was present, but functionally inactive. Cochrane, *et al.* subsequently reported that the inactivation of α_1PI in BAL fluids of these patients resulted from three causes: oxidation, complex formation with neutrophil elastase, and proteolytic cleavage of α_1PI (20). The portion of α_1PI oxidized but not cleaved (from molecular weight of 52,000 to 47,000) could be reactivated to neutralize elastase activity when, *in vitro*, it was subjected to reduction. The α_1PI circulating in the plasma was 90% active in 14/22 cases and between 50-90% active in the other eight patients with ARDS. The circulatory α_1PI in the plasma of seven normal individuals was between 71 and 100% in the active form. The finding of apparently adequate levels of active α_1PI in the circulation suggested that the α_1PI in the lungs of patients with ARDS became inactivated after entering the lung.

Other investigators have also found evidence of NE antigen in BAL from ARDS patients, but have failed to find evidence of NE activity (23-25). In these patients, NE was predominately complexed to α_1-PI, and excess active α_1-PI was present. A small fraction of NE (< 0.4%) was complexed to the 725 kD inhibitor, α_2-macroglobulin. Whether active NE is found in BAL from ARDS patients may depend on the clinical status of the patient and on sampling technique. Results of all studies indicate that NE is secreted from PMN in the lung, and may be available for proteolysis in the absence of inhibition by α_1-PI. Consistent with this view, elastin degradation products have been detected in serum and BAL of patients with ARDS (26).

Recent experimental evidence suggests that parenteral therapy with a partially purified preparation of α_1PI effected an elastase-antielastase balance within the lung of individuals with α_1PI deficiency and severe emphysema (27,28). Infusions of four grams of α_1PI intravenously at weekly intervals for four doses maintained serum levels at $\geq$ 70 mg/dL, the level likely required for effective antielastase protection of the lung in this chronic disease. No patient developed inhibitory antibodies to α_1PI at periods up to one year follow-up.

Recently, Cutter Biological, Inc. has developed a preparation of human α_1PI that is now approved for human use. Alpha-1-Proteinase Inhibitor (Human) is a sterile lyophilized preparation of partially purified human α_1PI is $\geq$ 0.35 units/mg protein and when reconstituted, the concentration of α_1PI is 25 $\pm$ 5 mg/ml. The total

protein content is 20-75 mg/mL. When reconstituted, α_1PI has a pH of 6.6-7.4, a sodium content of 100-210 mEq/L, a chloride content of 60-180 mEq/L, and a sodium phosphate content of 0.015-0.025 M. The osmolality is 20-400 m0smol/kg. A_1PI also contains small amounts of other plasma proteins including alpha-2-plasmin inhibitor, alpha-1-antichymotrypsin, C1-esterase inhibitor, haptoglobin, antithrombin III, alpha-1-lipoprotein and thus should be used immediately on reconstitution and entry.

Alpha-1-Proteinase Inhibitor (Human) is prepared from pooled human plasma of normal donors by a modification of the cold ethanol method of Cohn. Each unit of plasma used in the manufacturing process has been found non-reactive for hepatitis B surface antigen (HBsAG) using a U.S. federally approved test with third generation sensitivity. In addition, α_1PI has been heat-treated in solution at 60 ± 0.5 C for not less than 10 hours, in the presence of stabilizers during the manufacturing process prior to lyophilization. The administration of this material by intravenous infusion may offer new opportunities for treatment of ARDS. It remains to be determined, however, if α_1PI administered parenterally to the patient with ARDS will: (a) rapidly gain access to the microenvironment in which elastase and structural proteins including elastin, collagen, and fibronectin are in contact; and (b) remain active to modify and attenuate the clinical course of acute lung injury. The first part of the contract on which this report is based was designed to address those questions.

Abnormalities of the lung surfactant system in patients with acute lung injury:

Adult RDS bears some similarity to the clinical and biochemical features of infant RDS, or hyaline membrane disease of the newborn. In this latter disorder, the cause is understood to be surfactant deficiency; the alveolar type II cell, the major site of production of lung surfactant, has not matured to its full synthetic capacity. For both experimental and theoretical reasons, surfactant is believed to function to maintain low alveolar surface tension and prevent alveolar collapse with loss of lung compliance, and also to retard influx of water from the pulmonary interstitium (29,30). A fairly clear relationship exists between the deficiency of surfactant in the bronchial wash of infants with RDS and their derangements of gas exchange and lung mechanics (31,32). In these premature newborns, pilot studies show that giving exogenous surfactant through an endotracheal tube results in dramatic, though frequently transient, improvements in hypoxemia and lung compliance (33-36).

The major component of surfactant, phosphatidylcholine, consists mainly of dipalmitoylphosphatidylcholine (DPPC) and other disaturated fatty acids. Several components of lung surfactant, including DPPC and surfactant apoproteins B and C appear to be responsible for decreasing surface tension as the liquid-air interface at which surfactant is localized is compressed (as during expiration).

In pursuing the relationship between infant and adult RDS, it has been found that there are qualitative abnormalities in the composition of surfactant obtained from bronchoalevolar lavage (BAL) of patients with ARDS (Table 1). In particular, the fractional content of phosphatidylcholine, the phosphatidylcholine/ sphingomyelin ratio (an index of fetal lung maturity) and the % phosphatidylglycerol were much lower in a group of ARDS patients than in controls with other respiratory diseases (37). These changes resemble the pattern seen in infant RDS, and may be responsible for the poor surface activity of ARDS lung lavage *in vitro* (37). It is not yet known if there is actually a quantitative deficiency of BAL surfactant in ARDS relative to normal adults.

Whether surfactant abnormalities are part of the disease pathogenesis in ARDS is also an open question. Surfactant has not been found to be abnormal in ARDS victims just prior to the onset of respiratory failure (38). It is possible that surfactant abnormalities appear only secondarily in ARDS, due to the presence of inhibitor in the plasma leaking into alveoli. Indeed, supernates of BAL from premature lambs have been found to contain protein (39), and glycolipid inhibitors (40) of surfactant function, *in vitro* and *in vivo*. Such an inhibitor has been found in the BAL of ARDS patients as well (38). Substances known to have inhibitory function include glycolipid, albumin, hemoglobin, fibrin monomer, and bilirubin (41).

Despite uncertainty about the cause of the surfactant abnormalities found in patients with ARDS, it is reasonable to hypothesize that replacement therapy with intact surfactant might restore (at least temporarily) surfactant activity to the alveolar lining fluid. This therapy, if successful, would promote alveolar ventilation, increase pulmonary compliance, decrease shunt, and decrease the requirement for high and therefore toxic inspired oxygen concentrations.

Recent work discloses a biochemical pattern in infants with RDS similar to that found with ARDS. Approximately 30% of surviving infants with RDS go on to develop bronchopulmonary dysplasia, a progression of the acute alveolar inflammation to chronic interstitial cell proliferation and fibrosis with focal emphysema. These same infants exhibit high numbers of neutrophils and elevated elastase activity in the BAL early in their disease (d. 3- 14) associated with a reduced α_1PI activity (42). When RDS infants are treated with exogenous surfactant, they do not develop the same derangements of BAL elastase and α_1PI activity (43). It is likely that the biochemical parallels between adult and infant RDS exist simply as part of a common final pathway of inflammatory lung damage, rather than as indicators of similar etiologies. Nonetheless, this observation provides rationale for measurement of BAL elastase and α_1PI in patients with ARDS who undergo replacement therapy with surfactant.

The study of surfactant replacement therapy in lung disease of premature infants and of animals has answered some questions about the best route of administration and the type and quantity of surfactant needed. Both natural surfactant made from human amniotic fluid and bovine lung, and "artificial" surfactant made of combinations of the major phospholipids found in natural surfactant have been used. Artificial surfactant demonstrates good in-vitro surface activity (44) and has the theoretical advantages of lacking antigenic foreign protein and requiring a small instillation fluid volume. However, in-vitro results of artificial surfactant replacement in infant RDS have been clearly inferior to direct tracheal instillation of natural surfactants (45-48). This is probably due to the important role of surfactant apoproteins and other quantitatively minor components in promoting spreading and stability of the major surface-active lipids such as dipalmatylphosphatidylcholine (DPPC) and phosphatidylglycerol (PG). Recently, however, some investigators have shown that protein-depleted surfactant lipids are equally as good as whole surfactant in "spreading" and reducing bubble surfaced-tension *in vitro* (49), and are effective *in vivo* (human infants) as well (50).

A relatively large number of controlled and uncontrolled studies with exogenous surfactant have been reported in the literature. These studies have been of two basic designs, and all have addressed the use of exogenous surfactant in the neonatal setting. In "rescue" or treatment studies, surfactant is given to low birthweight infants with established RDS; in "prevention" studies surfactant is given shortly after birth to infants at hightest risk for RDS, e.g. those infants less than 30 weeks gestational age, in order to prevent or ameliorate the syndrome. The reported studies and the type and quantity of surfactant used are presented in Table 2

(treatment studies) and Table 3 (prevention studies).

In all of the controlled rescue studies except one in which a synthetic formulation was used (51), a single dose of surfactant was associated with significant improvement in oxygenation and ventilation. Two single dose studies (52,53) and one multiple dose study (54) reported significantly decreased mortality in surfactant treated infants. In the controlled prevention studies, decreased incidence of death and/or bronchopulmonary dysplasia was demonstrated in two single dose studies (55,56) and in one multiple dose study (57) Three studies reported decreased need for ventilatory support (55,57,58). Results of rescue and prevention studies suggest that more than one dose of surfactant replacement may be necessary to maximize the clinical response. No toxicity to surfactant replacement has been reported in any study.

Experience under controlled circumstances with administration of lung surfactant to patients with ARDS did not exist prior to this study. Lachmann used surfactant supplementation to treat several terminally ill patients with ARDS and found that intratracheal instillation of natural surfactant in a volume equal to the anatomical dead space was tolerated. No improvement in gas exchange was observed until a cumulative dose of greater than 200 mg/kg was administrered. In addition, he reported his first clinical results in a terminal patient with sepsis and ARDS. Four hours after tracheal instillation of natural surfactant (300 mg phospholipids/kg body weight), p_aO_2 improved from 19 torr to 240 torr (F_IO_2 = 1.0) and a chest radiograph taken four hours after instillation showed "nearly normal lung fields" when compared to an examination 20 minutes before treatment (59,60).

King and Clements (61) showed in dog experiments that 1.2 mg/gram wet lung weight would be sufficient to cover the alveolar surface with a surfactant monolayer (1 gram of surfactant for the 800 gram human lung). This could be regarded as a lower limit however for surfactant replacement in diseased lungs because of: (a) maldistribution, (b) transepithelial leak of material delivered into the airways, and (c) inactivation by surfactant inhibitors.

Observations by Ikegami *et al.* demonstrate that in premature lambs with RDS, the quantity of surfactant necessary to achieve maximal increase in $Pa0_2$ and lung compliance was 50-60 mg/kg body weight (62). A rough extrapolation to adult humans (70 kg) would suggest that a dose of 3.5 to 4.2 grams of surfactant may be adequate. This dose is in close agreement with that derived from previous work by Robertson. He used 250 mg to successfully treat premature infants with RDS whose wet lung weights are likely to have been approximately 50 grams. The human adult lung weighs approximately 800 grams, and therefore may be presumed to have an alveolar surfactant pool 16 times as great as infants. Thus, a calculated dose of 4 grams (16 x 250 mg) may be appropriate for an average sized adult.

Other considerations suggest that a dose of 4 gm surfactant / 70 kg adult may be an underestimate. In clinical trials, during surfactant administration infants were held in different positions to facilitate delivery to all lobes. Clearly this practice would be limited in adults with ARDS. It is possible that bronchoscopic delivery into each lobar bronchus provides more homogeneous delivery. Nevertheless, completely homogeneous distribution of the surfactant is unlikely given the regional lack of ventilation in diseased portions of ARDS lung. Recent studies have shown that homogeneity of distribution (in lungs of normal rabbits) is proportional to dose volume and independent of dose concentration. Problems with surfactant delivery have been studies in premature lambs (63). If the lambs were treated with intratracheal surfactant after the development of RDS, surfactant was found preferentially in aerated portions of lung, whereas blood flow was directed away from these aerated portions. This inhomogeneous delivery did not occur in lambs treated at birth. It has been found that treatment of

premature lambs before the onset of RDS results in a greater duration of benefit (8 hr.) than rescue of lambs with established RDS (3 hr.) (64). Presumably this effect is due to the presence of surfactant inhibitors that develop with the onset of disease. It is likely that a single dose in ARDS would have an effect of less than 12 hrs. duration.

Pharmacodynamic issues may also affect the duration of therapeutic effects. In studies of labelled surfactant precursors injected I.V., a simple precursor-product relationship does not exist between the type II cell and the alveolar pool (65,66). The explanation offered for this is a now well-established concept of bidirectional flux, whereby whole surfactant lipid given into the airways is taken up by lung epithelia and re-secreted intact (67). In fact only 10% (newborn rabbits) to 30% (adult rabbits) of alveolar surfactant is synthesized *de novo* (68,69). Release of surfactant from pulmonary epithelia is also affected by ventilatory pattern; the high minute ventilations in ARDS may significantly increase the turnover of surfactant (70).

All of these considerations make it difficult to arrive at any theoretically optimal intratracheal dose of surfactant for patients with ARDS. It is, however, noteworthy that the higher doses used in some clinical trials have not caused observable ill effects. Of particular concern, however, is the danger of immunization against surfactant proteins (71). Because we have used a heterologous (bovine) surfactant in this study, it was particularly important to search for the development of antibody directed against surfactant components, and/or the presence of immune complexes containing surfactant components. Immunization to xenogenic surfactant protein by intratracheal administration is unlikely, based on the marked difficulty in immunizing animals to foreign protein delivered via the airway. It should be noted, however, that the transcapillary leak of plasma in ARDS undoubtedly means that any protein instilled into the airways has abnormal accessibility to the blood compartment as well, making a systemic immune response conceivable. In fact, infants with RDS, treated with human amniotic surfactant, have been found to develop circulating immune complexes of surfactant and anti-surfactant antibody. However, no untoward clinical consequences have been apparent (71).

Specific aims:

Alpha-1-proteinase study:

1. Determine the safety of α_1PI administered intravenously to patients with ARDS;

2. Determine the dose response and half-life of α_1PI in serum after intravenous infusion of α_1-PI to patients with ARDS;

3. Demonstrate the passage of intravenously administered α_1PI across the alveolar membrane from the blood into the alveolar spaces;

4. Determine the antielastase activity in blood and bronchoalveolar lavage fluids after administration of α_1PI.

Surfactant study:

1. Determine if bovine surfactant, administered early into the airway of patients with established ARDS, modifies pulmonary gas exchange and compliance as measured by:

   a. Oygenation (a/A ratio and pulmonary shunt);

   b. Changes in total thoracic compliance at comparable lung volumes;

   c. Chest radiograph.

2. Evaluate in an uncontrolled fashion outcome with regard to the endpoints of:

   a. Length of ventilator dependence;

   b. Evidence of barotrauma (development of BP fistulae);

   c. Survival.

3. Obtain BAL sequentially for analysis of neutrophil elastase (functional and total), alpha-1-PI (functional and total), albumin and total and differential WBCs. These measurements may reflect the effect of surfactant therapy on modifying the acute inflammatory lung injury.

4. Assay for the development of antibody and circulating immune complexes to both bovine and human lung surfactant in the short term (15 days) and long term (1 mo, 6 mos.).

6. Body

A. α_1-PI studies

1. Patient protocol

a. Eligibility:

1. Patients of either gender $\geq$ 19 years of age who, in conjunction with sepsis, pancreatitis, thermal injuries, bacterial lung infections or trauma, develop acute respiratory distress and require ventilatory support are eligible if they:

(a) develop acute respiratory failure within seven days;
(b) have diffuse pulmonary edema as documented by roentgenogram; and
(c) maintain a pulmonary capillary wedge pressure of 18 mm Hg or less.

In order to be eligible, each patient must be able to understand any potential benefits and risks of enrolling in the study and submit a signed informed consent document.

2. The following will be ineligible for study:

(a) Any patient who does not fulfill the criteria of eligibility will be excluded.
(b) Patients with known pre-existing chronic lung diseases will be excluded.
(c) Patientwith a history of anaphylaxis to iodide salts and iodinated proteins as used in radiodiagnostics will be excluded.
(d) Women of childbearing potential will be excluded (unless they are surgically menopausal or a pregnancy test is negative within a week prior to the study).
(e) Patients receiving pharmacologic support to maintain blood pressure will be excluded.
(f) Patients with an endotracheal tube size < 7 French will not be enrolled in the study.l
(g) Patients who require a FI02 > 0.9 to maintain a $Pa0_2$ of at least 60 torr will be excluded.
(h) Individuals who, in the opinion of the investigators, have any contraindication to bronchoscopy and bronchoalveolar lavage will be excluded.
(i) Individuals will be excluded if they, or their designated adult relative, are unwilling to five informed consent.

b. Patient protocol: Overview

Patients who meet eligibility requirements and have received continuous mechanical ventilatory support for $\geq$ 60 hours with little or no evidence of improved pulmonary gas-exchange will be

enrolled. Each patient will receive thyroid prophylaxis with SSKI and begin an intense period of observation and evaluations to establish a baseline data base. Clinical and laboratory evaluations for the next 12 to 18 hour period will include frequent monitoring of vital signs including heart rate, blood pressure (systemic and pulmonary wedge pressures), body temperature, ventilator requirements, arterial blood gases, fractional urine collections, and hematologic and serum biochemical parameters. Each patient will undergo bronchoscopy and BAL during the observation period.

Upon completion of the baseline period (after $\geq$ 72 hours of continuous ventilator support) alpha-1-PI will be given intravenously at a dose of 60 mg/kg at a rate of 0.08 ml/kg/minute. The 125-I alpha-1-PI (300 μCi) will be given during the last five minutes of the alpha-1-PI infusion.

A series of blood tests and urine collections will be obtained following the infusion of alpha-1-PI. The tests and the frequency at which they will be obtained are outlined in Section 5.B.1-3. Repeat bronchoscopy and BAL will be done four to six hours after the alpha- 1-PI infusion is completed and again after another 24 to 30 hours post-infusion of alpha-1-PI. BAL effluent will be collected and analyzed as previously described. Serum chemistries, and the clinical course of the patient following the single infusion of alpha-1-PI will be obtained at defined intervals.

During the course of this study, the participants will continue to receive allmedications and other supportive care that they would receive if they were not participating in the study. No medications or other modes of therapy will be stopped or decreased in frequency because of the study.

c. Time Sequence

(1) Baseline measurements. Prior to the administration of alpha-1-PI, baseline observations obtained during the 12-18 hour period will include:

- Recording of history; physical examination; measurements and body temperature (systemic and pulmonary wedge pressures) obtained and recorded at least every four hours;
- 12 hour urine collection for quantitative determinations of desmosine and creatinine concentrations;
- Arterial blood gases obtained prior to the administration of alpha-1-PI;
- Alpha-1-PI phenotype;
- Alpha-1-PI serum levels, including:
  immunologic (immunoelectrophoresis)
  functional (elastase inhibitory activity)
  elastase - antielastase immune complexes (optional)
- Complete blood count and platelet count

- Serum biochemistry profile (Chemistry Panel III includes the following: glucose, BUN, creatinine, sodium, potassium, bilirubin, CPK, SGOT, SGPT, LDH, alkaline phosphatase, total protein, albumin and globulin, amylase;
- Chest radiography (portable)
- Fiberoptic bronchoscopy and BAL of right middle lobe or lingula. Effluent collected during BAL will be analyzed for the following:
  total volume
    albumin concentration
    alpha-1-PI concentration
      immunologic (immunoelectrophoresis)
      functional (anti-elastase activity)
    total and differential cell count
    elastase-antielastase complexes
    elastase

(2) At least two hours following bronchoscopy, alpha-1-PI will be administered.

- Alpha-1-PI will be reconstituted as directed and brought to room temperature.
- The alpha-1-PI, immediately after reconstitution, will be administered at a rate of 0.08 ml/kg/min. Each patient will receive 60 mg of alpha-1-PI/kg body weight.
- An additional small amount of alpha-1-PI that has been radiolabelled (300 μCi) with ^{125}I will be given as a "push" infusion with a following saline flush through an inline Y-connector infusion port during the last 5 minutes of the alpha-1-PI infusion.
- Vital signs (BP, temperature, pulse rate) will be monitored every 15' prior to, beginning, and during the administration of alpha-1-PI. In addition, pPA wedge will be monitored during the infusion and a CBC and platelet count will be obtained 5 and 30 minutes following the onset of the infusion.
- Preparation, handling and administration of radiolabelled alpha-1-PI. A portion of the alpha-1-PI provided by Cutter Biological, will be radiolabelled with 125-iodine by the lactoperoxidase method. Procedures used in radiolabelling alpha-1-PI and the storage, admixing and disposition of radiolabelled materials will comply with the Radiation Protection Committee of the University of California, San Diego. The absence of pyrogenicity of I-125 alpha-1-PI will be documented before use in compliance with the Radiation Protection Committee (rabbit tests). The radiolabelling of alpha-1-PI with 125-iodine alters neither its *in vitro* electrophoretic characteristics nor its elastase-inhibitory capacity.

(3) Studies to be obtained after the administration of alpha-1-PI.

- Upon completion of the infusion of alpha-1- PI, the following will be obtained (Day

1): Venous blood samples (2 cc) for the determination of serum alpha-1-PI levels (Immunological and functional assays). These blood samples will be obtained 15 minutes after completion of the infusion and every 15 minutes thereafter for the first hour, then every 30 minutes for the following 5 hours, then every eight hours to 24 hours and then daily for six days. Analyses will include the following: alpha-1-PI (immunoelectrophoresis); antielastase activity); radioactivity in whole blood, in plasma and, after TCA precipitation, in supernatant and precipitated material.

- Arterial blood gases obtained five minutes after alpha-1-PI and again before bronchoscopy at four to six hours after alpha-1-PI is given.
- Serial 12-hour urine collections for desmosine and creatinine will commence. Two 12-hour collections will be obtained.
- Bronchoscopy and bronchoalveolar lavage of a segment of the lobe previously lavaged will be obtained four to six hours after completion of the alpha-1-PI infusion. Analyses of the lavage effluent:
  - total volume
  - albumin concentration
  - alpha-1-PI concentration
    - immunologic
    - functional
  - total and differential cell count
  - radioactivity (before and after TCA precipitation)
  - elastase-antielastase complexes
  - elastase
- Day 2 (24-48 hours after alpha-1-PI administration)
  alpha-1-PI serum levels (immunological and functional)
  complete blood count
  bronchoscopy and BAL
- Day 3 (48-72 hours after alpha-1-PI administration)
  alpha-1-PI serum levels (immunological and functional)
- Day 4 (72-96 hours after alpha-1-PI administration)
  alpha-1-PI serum levels (immunological and functional)
  Chemistry Panel III
- Day 6 Alpha-1-PI serum levels (immunological and functional)
- Day 8 Alpha-1-PI serum levels (immunological and functional)
- Day 10 Alpha-1-PI serum levels(immunological and functional)

2. Bronchoalveolar lavage protocol: A fiberoptic bronchoscope is wedged into either the right middle lobe or lingular bronchus and a single bolus of 60 ml of 0.9% NaCl is instilled and immediately withdrawn by hand aspiration. The volume is recorded and the specimen is centrifuged at 200 x g for 10 minutes at 4°C. The cell pellet is resuspended and analyzed for white cell count and

differential and the supernatant is frozen at -20°C for later biochemical analyses.

3. Data Analysis:

a. The safety of alpha-1-PI will be determined by testing for significant changes from baselinehematology, blood chemistry and vital sign data, using analysis of variance techniques. In addition, adverse reactions will be monitored on a patient-by-patient basis.

b. The measured plasma radioactivity for each individual patient was fit to the biexponential equation: $CPM/ml = A*e^{(-\alpha t)} + B*e^{(-\beta t)}$ using non-linear least squares regression with statistical weighting for the residuals between data points and the calculated fit (72,73). The calculated parameters A, α, B, and β for each patient were used to calculate pharmacokinetic parameters using the following equations (74).

$$Volume_{(central)} = Administered\ dose/(A+B)$$

$$Volume_{(steady\ state)} = Administered\ dose/B$$

$$Half\ life_{(central)} = \ln(2)/\alpha$$

$$Half\ life_{(steady\ state)} = \ln(2)/\beta$$

$$Clearance = (Administered\ dose)/(A/\alpha + B/\beta)$$

c. The passage of alpha-1-PI across the alveolar membrane from the blood into alveolar spaces will be determined by comparing post-infusion amounts of alpha-1-PI in the lungs to baseline data using analysis of variance techniques. Changes in functional activity of alpha-1-PI and change in lung tissue degradation products will be tested for significance using repeated measure techniques for analysis of variance.

4. Experimental methods

a. α_1-PI iodination: Sepharose beads (0.025 ml) with lactoperoxidase covalently coupled to the surface are washed with 4 x 1 ml of phosphate buffered saline (PBS). 2.5 mCi of ^{125}I is then placed in a 1.5 ml tube microcentrifuge tube in a volume of 0.050 ml. Carrier iodide (50μM final) and 5.0 mg of highly purified α_1-PI (single band on SDS-polyacrylamide gel electrophoresis) are added and the volume brought to 1.0 ml with PBS. The reaction is initiated by the addition of H_2O_2 (0.10 mM final) and allowed to proceed for 30 min. The labelled α_1-PI is separated from the other reactants by elution over a PD-10 desalting column equilibrated with PBS, collecting sequential 0.5 ml fractions. Protein containing fractions are

pooled and sterilized using a 0.45 μm filter. The volume required to deliver 300 uCi to a patient is calculated and 5% of this anticipated volume is administered parenterally to each of three rabbits for pyrogen testing. Rabbit rectal temperatures are recorded prior to injection and hourly for 5 hours after injection.

Summary of labeling procedures:

Batch		#1	#2	#3	#4	#5
Volume pooled fractions (ml)		2.585	2.07	2.557	2.427	1.940
Radioactivity	(μCi)	1830	1250	1534	1031	810
	(μCi/ml)	708	604	600	425	418
Pyrogen testing:		neg.	neg.	neg.	neg.	neg.

We have demonstrated rigorously that this labeling procedure does not inactivate α_1-PI (75).

b. Plasma analyses: Samples of blood are collected in EDTA containing tubes and placed on ice immediately after collection. The sample was spun at 1500x g for 10 minutes and the plasma supernatant removed. Individual aliquots of plasma are then stored at -70° C until subsequent analysis.

(1) α_1-PI level by immunoelectrophoresis: 22 ml of 1.0% agarose gel in 0.02 M barbital buffer buffer (pH 8.6) (20 wells/plate) is prepared for each 1.5x 110 x 125 mm plate. For samples with low levels of α_1-PI, polyethylene glycol (3%) is added to the agarose to promote aggregate formation. After dissolving agarose and polyethylene glycol over a boiling water bath, the mixture is allowed to cool to 55-58° C. Antibody (IgG fraction of goat antihuman α_1-PI; Cappel, Inc.) is added to 0.30% v/v and the molten solution is poured evenly onto a Gel Bond plate. After gelation, the plate is stored at 5° C until use. To perform the electrophoresis, 2.5 mm holes are punched in the agarose and the plugs are removed by gentle aspiration. The plate is placed into a Bio-Rad Model 1400 electrophoresis chamber using 0.02M barbital buffer (pH 8.6) as the electrode buffer, and 100 V applied for 10 hours. Following electrophoresis, the gel is dried, stained with Coomassie Blue dye, and decolorized. The height of each α_1-PI rocket is measured to the nearest 0.1 mm with a magnifying eyepiece, and a linear regression analysis of the relation between standard concentration and rocket height performed.

Plasma samples are typically diluted 1:200 in saline and BAL samples are usually run undiluted or diluted 1:5 in PBS. The standards are prepared from purified α_1-PI (CalBiochem, La Jolla) and used at concentrations from 20 to 80 ug/ml.

(2) α_1-PI activity: Functional α_1-PI activity is assayed by examining the ability of samples to inhibit purified porcine pancreatic elastase. The elastase is prepared by diluting a stock elastase preparation (2.0 mg/ml) into Tris buffered saline (Tris 0.1M pH 8.0, NaCl 0.15 M) containing 1.0 mg/ml bovine albumin (TBS/BSA). A working solution of the elastase substrate, N-succinly-L-alanyl-L-analyl-L-analyl-p-nitroanilide (SLAPNA), is prepared from a stock solution (200 mM in pyrrolidinone) by dropwise addition to room temperature TBS. Solutions of 1mM and 10 mM are prepared for analysis of plasma and BAL samples respectively. For preparation of a standard curve, purified α_1-PI (commercially available from CalBiochem, La Jolla, CA, and active site titrated against neutrophil elastase in our laboratories) is diluted with TBS/BSA. Standards containing up to 16 μg/ml (for analysis of plasma samples) or up to 5 μg/ml (for analysis of BAL samples) are prepared for each assay. Samples are diluted 1:200 in TBS/BSA (plasma) or analyzed without dilution (BAL).

To perform the assay, pancreatic elastase (50 ul containing 333 ng for plasma samples or 100 μl containing 83 ng for BAL samples) is placed into the wells of a 96 well plate. Samples or standards (50 ul/well for plasma or 100 μl/well) are added to each well and allowed to incubate with elastase for at least 15 minutes with agitation. Substrate (100 μl of 1.0 mM or 10μl of 10.0 mM SLAPNA) is then added to each well, and the reactions are allowed to proceed for 15 minutes (plasma samples) or 30 min (BAL samples). The reaction is stopped by the addition of 10 μl of 10N acetic acid. After the addition of acetic acid, the difference between absorbance at 405 nm and 595 nm is read on the VMax microplate reader. Sample concentrations are calculated from a 4-parameter quadratic equation fit to the plot of standard concentration vs absorbance.

(3) α_1-PI radioactivity: Aliquots of plasma, 0.5 ml, are diluted with an equal volume of 20% trichloroacetic acid (diluted volume/volume in water), vortexed, and incubated for 30 min on ice. The samples are centrifuged at 10,000x g for 10 min and 0.5 ml of supernatant removed. Measured aliquots of plasma (0.5 ml), TCA supernatant (0.5 ml), and BAL (1.0 ml) are counted for 10 minutes in a gamma radiation counter along with a diluted sample of the injected radioiodinated A_1-PI, and the resulting counts corrected to CPM/ml of original sample.

c. Bronchoalveolar lavage analyses

(1) Cell count: Samples are received in the laboratory on ice. The sample is filtered using sterile technique by placing a small piece of gauze (two layers) which has been dampened with cold sterile saline in a small funnel in a 50 ml centrifuge tube. The BAL fluid is expelled from the syringe through the gauze and into the centrifuge tube. The volume is measured and approximately 50 μl of fluid is removed with a sterile pipet tip and placed

in a 12 x 75 polystyrene tube. An equal volume of trypan blue dye is added and tube is incubated for 5-10 minutes. Cell number is determined using a hemocytometer, and the fraction of cells excluding trypan blue is recorded. The remaining BAL is spun at 500 g for 15 minutes and the supernatant is saved for subsequent analyses.

(2) Cell differential: The volume of BAL fluid containing 1 x 10^5 cells is calculated, and that volume is placed in a flat bottomed centrifuge tube over a circular 12 mm coverslip. The tube is centrifuged for 15 minutes at 500 x g. The coverslip is dried, stained with DiffQuick stain, and mounted on a glass slide. At least 100 cells/slide are counted, noting the percentage of polymorphonuclear leukocytes, lymphocytes, and macrophages.

(3) α_1-PI level - See 6. A. 1. 4. b. (1)

(4) α_1-PI activity - See 6. A. 1. 4. b. (2)

(5) α_1-PI-HNE complex level: 96 well plates (Immulon II; NUNC, Inc.) are coated with the first antibody (purified rabbit IgG against human neutrophil elastase raised in our laboratories) diluted 1:10,000 with 0.05 M NaHCO3 buffer, pH 9.6. 100 µl of the diluted antibody is added to each well, the plate is covered with plastic plate sealer (Fisher #14-245-18) and incubated in a moist chamber at 4 °C overnite. Wells are then washed with the same $NaHCO_3$ buffer and unoccupied binding sites on the plate blocked by the addition of $NaHCO_3$ buffer containing 10 mg/ml bovine serum albumin (RIA grade). The plates are incubated at room temperature for 2 hours in a moist chamber and then washed twice with PTB (20 mM NaH_2PO_4, 150 mM NaCl, 0.06% Tween 20, 0.10% BSA). To measure complex levels, 100 µl of sample or standard is added to each well and the plate is incubated in a humidified chamber for 1 hour. Wells are washed twice with PTB to remove any sample not bound to the plate. Second antibody (100 µl of peroxidase conjugated goat anti-human α_1-PI IgG (Cappel) diluted 1:4,000 in PTB) is added to each well and the plate is incubated in a humidified chamber for one hour. Each well is then washed twice with PTB and 100 µl of peroxidase substrate (0.5 M citric acid, 0.11 M Na_2HPO_4, 10% citric, 1 mg/ml OPD, 1 µl/ml 30% H2O2) is added. The absorbance of each well is read at 490 nm after the reaction is terminated by the addition of 100 µl of 3N H2SO4/well, and a curve of best fit is calculated by microcomputer using a 4-parameter quadratic equation.

Standards are prepared by combining purified active site titrated human neutrophil elastase (HNE) with an excess of α_1-PI, and the predicted levels compared with a commercially available standard (Merk, Darmstadt). Dilutions of standards with PTB are prepared covering the range 1.25 - 20 ng/ml. A control sample containing a known concentration of complex is added as an internal standard on each plate.

The assay has been validated by analysis of standards present in a variety of concentrations of α_1-PI or of plasma.

(6) HNE activity: Samples or standards (100 μl) are added to each well of a microtiter plate. Substrate (200 μl of 0.5 mM methoxysuccinly-L-alanyl-L-alanyl-prolyl-L-valyl-p-nitroanalide kept as a 100 mM stock in methyl-2-pyrrolidinone and diluted in tris/NaCl buffer) is added to each well and the plate is incubated at 37°C for two hours. Developed color is read as the A405 - A570 difference on the VMax microtiter plate reader.

Standards are prepared from active site titrated leukocyte elastase (Elastin Products) purified from human sputum. Dilutions are made with tris/BSA buffer to produce concentrations from 10 to 200 ng/ml.

(7) α_1-PI radioactivity: See 6. A. 4. b. (3)

(8) Albumin concentration by immunoelectrophoresis: A 1% agarose gel in barbital buffer is prepared containing anti-human albumin (0.4% v/v of goat anti-human albumin, Cappel) as decribed in 6.A.4.b.(1). Plasma samples are diluted 1:1000 in PBS and BAL samples are run either undiluted or diluted 1:5 with the same buffer. Standards are diluted in PBS to final concentrations between 10 and 70 μg/ml. Samples or standards, 5 μl / well, are applied and electrophoresed at 100 V for 10 hours. Subsequently, the gel is dried, stained with Coomassie Blue dye and decolorized until clear. The height of each rocket is read to the nearest 0.1mm using a magnifying eye piece. Sample concentrations are read from a least squares fit to the plot of standard concentration vs. rocket height.

5. Results

a. Patient Enrollment: Seven patients were enrolled into and subsequently completed the study. A number of additional patients were evaluated for entry but could not complete the study because of hemodynamic instability (an exclusion criterion) or because of inability to tolerate bronchoscopy. The physiologic parameters at the time of entry into the study are summarized in Table 4.

(1) Brief summary of the medical history of each enrolled patient:

#1 FV, was a 64-year-old woman with a past history of diabetes mellitus, hypotension, and degenerative arthritis. She presented to UCSD Medical Center with septic arthritis progressing to frank staphylococcal septicemia. Hypoxemia and bilateral infiltrates on chest x-ray developed on the third hospital day and required intubation and mechanical ventilation by the seventh hospital day. There was no improvement in pulmonary function over the next two weeks and the patient was enrolled in the study on the 21st hospital day, receiving an infusion of α_1 antitrypsin the following day. There were no changes in vital signs during or immediately following the infusion. There appeared to be a transient improvement in oxygenation from 36 to 72 hours after the infusion but then progressive deterioration in gas exchange occurred over the next ten days accompanied by increased peak inspiratory pressures. At the request of the patient's family, life support was discontinued on the 33rd hospital day.

#2 BZ is a 48-year-old Hispanic male with a long history of intravenous drug use and of a seizure disorder following a heroin overdose 15 years prior to admission. The patient was admitted with fever and found to have pneumococcal pneumonia involving most of the left lung. Diffuse pulmonary infiltrates developed rapidly and progressed to severe respiratory distress syndrome. He had a hectic ICU course with minimal improvement in his respiratory function in the four weeks following his admission to the hospital. He was entered into the study on the 29th hospital day and received an infusion of α_1 antitrypsin on the next day. No change in his vital signs occurred during or immediately following this infusion. Study data was collected without incident and the patient subsequently had slow improvement in his pulmonary function culminating in removal from the ventilator on the 105th hospital day.

#3 TS is a 39-year-old woman with a long history of intravenous drug use. She was admitted to UCSD Medical Center with fever and hypotension. She was found to have staphylococcal sepsis causing disseminated intravascular coagulation and precipitating ARDS within 24 hours of admission. She had a stormy ICU course but stabilized after

4-5 days. She was then stable without clear improvement in pulmonary function and was enrolled in the study on the tenth hospital day with an infusion of α_1 antitrypsin on the following day. There was no change in vital signs during or immediately following the infusion. Over the subsequent weeks, the patient had a slow improvement in gas exchange and was successfully weaned from ventilator support and discharged from the hospital on the 27 hospital day.

#4 DC was a 44 year old woman with a long history of ethanol abuse and prior hospitalizations for GI bleeding, abdominal stab wounds, and hysterectomy for carcinoma *in situ*. She was admitted to UCSD Medical Center with pneumococcal pneumonia and sepsis. Her respiratory status deteriorated rapidly and required intubation during the first hospital day. She developed severe respiratory distress syndrome over the next four days manifested by increasing F_iO_2 requirements, diffuse pulmonary infiltrates, and the need for PEEP and therapeutic paralysis in order to maintain an adequate P_aO_2. After a period of stabilization of approximately one week, the patient had minimal further improvement in gas exchange over the next 7 weeks. She was enrolled in the study on the 63rd hospital day and received an infusion of α_1 antitrypsin the following day. There were no changes in vital signs with the infusion. There was a modest improvement in gas exchange over the four days following the infusion which allowed the withdrawal of therapeutic paralysis five days after infusion. Unfortunately, over the next two weeks the patient was found to have progressively deteriorating neurologic function with a CT scan showing bilateral cortical infarcts thought to result from septic embolization. Because of her deteriorating neurologic function the patient expired on the 82nd hospital day.

#5 LP was a 27 year old woman with a prior history of intravenous methamphetamine use who was admitted in labor to UCSD Medical and delivered a normal full term infant. The patient developed fever and pulmonary infiltrates over the 24-48 hours following delivery and was intubated for hypoxemic respiratory failure on the 3rd hospital day. A PA catheter was placed and confirmed the presence of culture-negative septic shock with a systemic vascular resistance of 419 dyne-sec/cm^5. Her ICU course was marked by increasing f_iO_2 requirements, multiple pneumothoraces, and a need for alpha adrenergic agents to maintain a stable blood pressure. By the 10th hospital day, the hemodynamic instability had moderated, pressor agents were no longer required, and the patient was enrolled in the study. An infusion of α_1 antitrypsin was adminisistered on the 9th hospital day with no untoward effects. There was no apparent alteration in vital signs or in pulmonary function following the infusion and, over the next weeks, the patient had a recurrence of sepsis physiology and required the reinstitution of adrenergic agents to maintain blood pressure. The patient expired of progressive hypotension on the 36th hospital day.

#6 JO is a 37-year-old white female with a history of ethanol abuse and Laennec's cirrhosis who was admitted to UCSD Medical Center for GI bleeding and hypotension. The patient required a transfusion of 6 units of packed red cells as well as multiple units of fresh frozen plasma. She was found to have increasing bilateral pleural effusions and subsequental developed hypercarbic respiratory failure requiring intubation on the second hospital day. Over the next three days the patient developed bilateral interstitial and alveolar infiltrates on chest film as well as progressive hypoxemia. A diagnosis of ARDS was made when the pulmonary capillary filling pressures were found to be low, and the patient was enrolled in the study on the 11th hospital day with an infusion of α_1 antitrypsin on the next day. There were no changes in vital signs during or immediately following the infusion, but there were decreasing F_IO_2 requirements over the next six days which allowed extubation on the 20th hospital day and discharge from the hospital on the 24th hospital day.

#7 CM is a 29 year old black male admitted to UCSD Medical Center for management of streptococcal sepsis felt secondary to intravenous drug use and forearm cellulitis. The patient deteriorated rapidly and required intubation within 48 hours of admission because of progressive bilateral pulmonary infiltrates and hypoxemia. His initial ICU course was complicated by the development of pyogenic arthritis secondary to hematogeneous disemination of bacteria, intermittent hypotension requiring adrenergic agents, and pulmonary barotrauma requiring multiple chest tubes. The patient stabilized with severely disordered pulmonary function over the three weeks following intubation and was entered into the study on the 26th hospital day with an infusion of α_1 antitrypsin the next day. There were no change in vital signs coincident with the infusion, but the patient had a worsening in oxygenation over the week following α_1 antitrypsin administration requiring a transient increase in f_iO_2 to 1.0. There was then a slow improvement in lung function over the next two months finally allowing the removal of ventilator support and discharge from the hospital on the 145th hospital day.

(2) Safety: Vital signs were examined every 15 minutes during the infusion and for the first hour following its administration. There were no alterations in blood pressure, heart rate, core body temperature, or respiratory rate attributable to the infusion. Serum chemistries and a complete blood count (CBC) were recorded during the baseline observation period, and at 24 hours following α_1-PI infusion (CBC) or 96 hours following infusion (chemistries). No significant alterations were noted in any of the measured parameters between the baseline values and those measured after the infusion (see Tables 5 and 6). In addition, in four patients, a CBC was obtained at the conclusion of the α_1-PI infusion to look for any immediate change in blood cell populations. There were no significant changes in platelet count, cell count, or cell differential associated with the infusion (data

changes in platelet count, cell count, or cell differential associated with the infusion (data not shown).

b. Plasma analyses:

(1) α_1-PI plasma levels: α_1-PI levels were elevated prior to infusion in all of the patients in the study (4.34 ± 0.61 mg/ml). Following the infusion of 60 mg/kg of plasma-derived α_1-PI, levels rose by 1.22 ± 0.15 mg/ml and then slowly fell to preinfusion levels (see Figure 1 and data for individual patients in Table 7).

(2) α_1-PI activity: In the preinfusion samples, the elastase inhibitory activity of the plasma, expressed as mg of α_1-PI equivalent inhibitory activity per mg of antigenically detectable α_1-PI, was 0.95 ± 0.03, suggesting that the bulk of the circulating α_1-PI was active and not oxidatively inhibited. After the infusion, the specific elastase inhibitory activity rose in parallel with the levels of antigenically detectable α_1-PI (Figure 2).

(3) α_1-PI pharmacokinetics: The increment in α_1-PI levels above the preinfusion value were fit to a single compartment model (74). The levels of detectable α_1-PI in plasma could be shown to decay with a $T_{½}$ of 37.9 ± 6.4 hours, and to have distributed into a cental compartment with a volume of 51.2 ± 3.0 ml/kg body weight. Both of these values are comparable to those calculated using plasma radioactivity.

α_1-PI-associated plasma radioactivity was measured at frequent intervals after the administration of the α_1-PI. Data from individual patients are shown in Table 8 and the analyses of those results is shown in Table 9. Composite data are shown in Table 10. The levels fell in a biphasic manner consistent with administration into a central compartment, diffusion into a steady-state volume of distribution, and subsequent clearance from the central compartment. The measured levels of radioactivity were fit to a biexponential decay equation and the calculated variables were used to estimate α_1-PI pharmacokinetic parameters (see Table 11). The pharmacokinetic behavior of α_1-PI in these ARDS patients was significantly different from that observed in individuals with stable emphysema (with or without congenital α_1-PI deficiency). The half life for distribution of α_1-PI from the central compartment and into the steady state volume of distribution, and the steady state half life were significantly shorter than the values measured in non-ARDS patients (see Table 12). In contrast, the central volume and the steady state volume of distribution were not different for ARDS versus non-ARDS patients. The shortened half lives, in combination with unchanged distribution volumes, resulted in a calculated rate of clearance of α_1-PI in ARDS patients that is threefold greater than the rate seen in non-ARDS patients ($p < 0.002$, Table 12). Examination of the residual radioactivity in plasma samples after precipitation with trichloroacetic acid showed that 98.1 ± 0.1% of the radioactivity

present was precipitable. There was no difference in the fraction of precipitable radioactivity between samples obtained at early or late time points, suggesting that, as expected, radioiodinated α_1-PI was stable during the period of this study.

c. Bronchoalveolar lavage analyses: Of the instilled bronchoalveolar lavage volume, 46.4 ± 1.8% was recovered (see Tables 13 and 15 for individual values).

(1) Cell counts: 2.24 ± 0.64 x 10^7 leukocytes were present in the pooled lavage fluid; there was wide variation between samples, but no significant changes were present between samples obtained in the baseline period, at 4-6 hours, or at 24 hours after α_1-PI infusion (Table 15).

(2) Cell differential cell counts performed on the lavage samples revealed the presence of increased neutrophils (41.0 ± 6.1%), and decreased macrophages (63 ± 6.4%). No significant differences were present in samples obtained at any of the three time points.

(3) α_1-PI levels in recovered lavage fluid varied widely depending on the adequacy of the lavage sample and the amount of dilution introduced during recovery of lavage fluid. When expressed as a ratio to the recovered lavage albumin, this variability was reduced. The levels of α_1-PI measured by an antigenic assay are shown in Figure 11. At baseline, the α_1-PI levels in these ARDS patients were 0.404 ± 0.096 μg/μg albumin and did not change significantly after the infusion of α_1-PI.

(4) α_1-PI-associated elastase inhibitory activity of the lavage fluid obtained prior to infusion was 73 ± 13% of the level of antigenically detectable α_1-PI in the same sample; there was no significant change in the specific α_1-PI activity after the infusion of α_1-PI. There was, however, wide variation in the in the α_1-PI-associated antielastase activity of the recovered lavage, with seven of 20 lavage samples containing α_1-PI which was 35% or more inactive. The levels of antigenically detectable α_1-PI in lavage fluid were similar to those in plasma when the plasma α_1-PI was normalized to the albumin level in the same sample ($p=.65$, see Table 14).

(5) α_1-PI in complex with neutrophil elastase was assayed in recovered lavage fluid through the use of a sensitive ELISA assay, and the measured levels were normalized to the amount of albumin in the same specimen. At baseline, the level of α_1-PI elastase complex was 3.86 ± 1.88 ng/μg albumin; After α_1-PI infusion, the BAL α_1-PI elastase complex levels were 9.06 ± 3.78 and 7.72 ± 2.78 ng/μg albumin at 6 and 24 hours respectively. These differences, although suggestive, did not meet statistical significance ($p=0.07$ and 0.064 respectively). In contrast to lack of difference between antigenic α_1-PI levels in BAL and plasma, lavage levels of α_1-PI elastase complex were substantially greater than the levels

simultaneously measured in plasma at each of the three time points ($p \leq 0.001$, Table 14).

(6) HNE activity: Consistent the finding of active α_1-PI in each sample detected, HNE activity was not detected.

(7) α_1-PI radioactivity: Lavage fluid radioactivity levels were used to calculate the amount of the *infused* α_1-PI which was present in the recovered lavage fluid. These analyses showed that 0.104 ± 0.028 and 0.083 ± 0.052 μg α_1-PI/μg albumin were present at 6 and 24 hours after α_1-PI administration respectively. Thus the lavage radioactivity levels would suggest that $51.3 \pm 16.3\%$ and $28.1 \pm 14.4\%$ of the lavage fluid α_1-PI originated from the infusion at 6 and 24 hours respectively. For this analysis, lavage samples with radioactivity levels less than three time background were excluded. The same samples that were excluded were also the most dilute by protein assay and contained the least levels of antigenic α_1-PI (typically ≤ 5.0 μg/ml).

(8) Albumin concentrations are shown in Tables 13; total protein values are shown in Table 15.

3. Discussion

The rationale for the administration of α_1-PI to patients with ARDS rests on a number of observations detailed earlier in this report. An influx of neutrophils into the lungs of patients with ARDS brings a full complement of proteolytic enzymes contained within intracellular granules; granules whose contents may subsequently be released into the local environment. These enzymes (*e.g.* neutrophil elastase) have been shown to have the capacity to degrade lung matrix proteins (76) and to hydolyze inflammatory mediators to active forms (77) and may, therefore, cause direct lung injury as well as participating in a cascade of pro-inflammatory events. In addition to being present in greater numbers, neutrophils in the lungs of patients with ARDS may also be more prone to release their granular contents. Cohen *et al* have shown that a macrophage-derived product that they have called enzyme-releasing peptide is found in significantly greater concentrations in the lungs of the patients with ARDS than in those with controls (78). This peptide has the potential to cause the release of neutrophil proteases from the cells that have migrated into the lungs. As well as an increased burden of proteases, particularly neutrophil elastase, the lungs of patients with ARDS may also have local abnormalities in the antiprotease defenses. These defenses rely primarily on the presence of functioning α_1-PI. Unfortunately, in the lungs of patients with ARDS, the α_1-PI may be oxidatively inactivated (21,22) or unable to function because it is in complex with neutrophil elastase or has been cleaved (77). The existence of preparations of concentrated semi-purified α_1-PI now allows therapeutic protocols designed to augment the anti-protease defenses of the lower respiratory tract, and will facilitate the testing of the hypothesis that uninhibited protease

activity is leading to enhanced lung injury. However, in order to design such protocols or to administer α_1-PI in a rational manner to patients, it is esential to understand the kinetics of transport of α_1 antitrypsin into and out of the acutely inflamed lungs of patients with ARDS. A description of these transport characteristics, an examination of the antiprotease activity of the infused α_1-PI, and a preliminary assessment of safety are the major emphases of this report.

The patients with ARDS who received α_1-PI as part of this study do not represent the entire spectrum of individuals who are afflicted by ARDS. For the most part, they had bacterial infections as the predisposing factor for ARDS rather than other etiologies (*e.g.* trauma or gastric aspiration). Furthermore, since the protocol was designed to examine individuals who would survive though the course of the study, all of the patients were past the early acute phase of ARDS and had been intubated for at least 8 days. However, the degree of impaired lung function demonstrated by a mean lung compliance of 14.1 ± 1.7 ml/cm H_2O (normal>50) and a P_aO_2/f_iO_2 ratio of 184 ± 26 mm Hg (normal >350) suggests that the individuals we studied did have severe ARDS. This conclusion is born out by the fact that 43% of the patients did not survive their ARDS even though they were selected for "stability". Thus, we believe that the individuals studied are reasonably representative of ARDS as a whole.

Earlier investigations have suggested the safety of α_1-PI administration to patients with emphysema (27,79,80). The data from this study would suggest that this conclusion can also be extended to patients with ARDS. No alterations in vital signs occurred during or immediately following the α_1-PI infusion. As might be expected during the administration of this relatively small volume of fluid (≈2 ml/kg), there were no detectable alterations in left ventricular filling pressures. Furthermore, there were no significant changes in hematocrit, in platelet count, or in white cell count or differential associated with the α_1-PI administration. Over the five day interval we monitored there were no demonstrable changes in any serum chemistry suggesting a change in renal or hepatic function. These observations are consistent with prior data describing the use of plasma-derived α_1-PI.

The concurrent bolus administration of radioiodinated α_1-PI with the the infusion of α_1-PI allowed precise measurement of α_1-PI kinetics in these ARDS patients. The central volume of distribution was unchanged in comparison with control (emphysema) patients. This finding is not unexpected since the central volume of distribution of α_1-PI is probably identical to the plasma volume. ARDS is often associated with peripheral edema formation which is usually attributed an expansion of the interstitial volume and "third spacing". The steady state volume of distribution for α_1-PI was somewhat larger in ARDS patients than in the controls, but this difference was not statistically significant ($p=0.09$). It is possible that studying patients earlier in their disease course, or studying a larger number of patients might show a significant alteration in the steady state volume of distribution. Regardless of any alterations in distribution volumes, the pharmacokinetic analyses in these patients demonstrates substantial alterations in the behavior of α_1-PI. The movement of α_1-PI

out of the central volume occurred with a half life 50% shorter than that of a control patient population. The surface area that this movement takes place across is thought to be identical to the vascular and capillary endothelial surface and it is not likely to be substantially different in size in ARDS patients. Therefore, it is likely that this shortened transit time is a reflection of enhanced permeability for α_1-PI across the endothelium. Movement of α_1-PI is thought to result from passive diffusion and the increase in permeability for α_1-PI is likely to apply to all plasma proteins of similar size (52,000 daltons). Somewhat more surprising were the alterations that were found in ß, the rate constant describing the steady state disposition of α_1-PI out of the central compartment. The T½ describing this disposition was decreased by 60% compared to control patients. The change in both half times plus the lack of change in the volumes of distribution for α_1-PI lead to a calculated value for the clearance of α_1-PI in ARDS of 0.816 ± 0.057 ml kg^{-1} hr^{-1}; a clearance which is one third that found in control patients. These data suggest that metabolism of α_1-PI is substantially enhanced in ARDS and they have important implications for dosing intervals during the therapeutic administration of α_1-PI.

The basal levels of α_1-PI were substantially elevated in the patients studied under this protocol. Since α_1-PI is known to be an acute phase reactant, this finding in not unexpected. The infusion of 60 mg/kg of plasma-derived α_1-PI resulted in a mean increment α_1-PI plasma levels of 1.24 ± 0.09 mg. There was a corresponding increment in the levels of plasma anti-elastase activity demonstrating that the infused α_1-PI retained all of its functional activity. The kinetic parameters derived from the antigenic α_1-PI measures were obtained after a slow infusion rather than a bolus injection, and so are not susceptible to detailed modeling. Furthermore, it is not possible to use the antigenic measures to calculate clearance of α_1-PI since we do not have any direct measure of daily α_1-PI synthesis. However, the steady state T½ of 37.9 hours and the volume of distribution measured by the antigenic assays are in close agreement with those calculated using plasma radioactivity and suggest that the radiolabelled α_1-PI and the infused α_1-PI behaved similarly.

The lavage fluid levels of α_1-PI were elevated in the preinfusion samples, but this elevation was identical to that in plasma when both levels were expressed as a ratio with albumin. There was not a measurable increase in the BAL levels of α_1-PI after the infusion, despite the fact that increased BAL radioactivity levels demonstrated passage of the infused α_1-PI into the lung. The failure to demonstrate an increase in α_1-PI is likely to be due to the fact that basal BAL levels of α_1-PI were elevated and the infused α_1-PI only augmented plasma levels by 28%. Given the marked variability in preinfusion plasma levels of α_1-PI and the inherent variability induced by dilution when lavage samples are obtained, it is not surprising that we could not detect significant changes in BAL α_1-PI. The observation that lavage levels of the infused α_1-PI were a substantial part of the total α_1-PI by the time of the 6 hour sample suggests that the same processes that led to enhanced transport of α_1-PI out of the central compartment and to enhanced central clearance of α_1-PI, have also altered transport of α_1-PI into the alveolar space. Specifically, the calculated level of infused α_1-PI in the BAL would suggest that plasma and BAL had largely reached equilibrium by the time the 6 hour

sample was obtained. This suggests that administration of α_1-PI directly into the alveolar space as an aerosol will also require a significantly reduced dosing interval in order to maintain therapeutic α_1-PI levels.

The functional activity of the lavage α_1-PI did not change after the infusion. Since the calculated contribution of the infused α_1-PI to those levels is significant, this observation would suggest that the α_1-PI that did reach the alveolar space retained its activity. A more precise calculation of activity is not possible given the variability in lavage α_1-PI levels. The specific α_1-PI activity in the lavage fluid was significantly less than that in plasma. This observation supports the hypothesis that inactivation of α_1-PI in ARDS takes place in the lung due to oxidative processes or to complex formation with elastase. Although we did not directly measure the presence of oxidatively inactivated α_1-PI , the levels of α_1-PI elastase complexes were not great enough to explain the loss of α_1-PI activity. Levels of α_1-PI elastase complex were, however, markedly greater in lavage fluid than in plasma and these differences suggest that elastase is being released locally into the lung in ARDS. Although there were no consistent alterations in plasma levels of α_1-PI elastase complexes before or after infusion of α_1-PI, in 5 of 7 patients there was a two-fold or greater change in complex level over a 24 hour or shorter period. This observation would suggest that elastase release (possibly into the lung) is episodic. It is not possible at the present time to predict when such a release might occur and therefore the results of assays of lavage fluid for elastase or for α_1-PI elastase complex levels are likely to be quite variable and may understate the magnitude of the elastase burden at any given time. This further supports a therapeutic approach aimed at providing consistently elevated lung α_1-PI levels of sufficient magnitude that any local release of protease could be overwhelmed. If the release of proteases is a local phenomenon, then augmentation of α_1-PI might be optimally performed by local administration as well. Technology exists for aerosol administration of α_1-PI and our data support such use in ARDS.

B. Surfactant studies

1. Patient protocol

a. Eligibility:

1. All patients in the Medical Intensive Care, Burn or Trauma Units of UCSD Hospital developing ARDS of any cause will be entered into the study as soon as possible (within 48 hours) following diagnosis of that syndrome. ARDS is defined by the following criteria:

(a) develop acute respiratory failure within seven days;
(b) have diffuse pulmonary edema as documented by roentgenogram; and
(c) maintain a pulmonary capillary wedge pressure of 18 mm Hg or less.

In order to be eligible, each patient must be able to understand any potential benefits and risks of enrolling in the study and submit a signed informed consent document.

2. Patients will be excluded for any of the following reasons:

(a) Known pre-existing chronic lung disease including pulmonary manifestations of allergy.
(b) Women of childbearing potential (unless a pregnancy test is negative within a week of the study).
(c) A.I.D.S.
(d) Any process which contraindicates bronchoscopy in the opinion of the investigator, or the primary physicians.
(e) Tracheal or esophageal injury or bronchopleural fistula.
(f) Endotracheal tube size < 7 mm I.D.
(g) Inability to obtain informed consent.
(h) History of allergy to porcine products.
(i) Positive skin test reaction to surfactant.

b. Patient protocol: Overview

The safety, and short-term clinical effect of bovine surfactant administered intratracheally to ARDS patients will be examined. This is a prospective double-blind, crossover trial of its short term effects and a pilot study of its long term therapeutic effects.

Each patient will serve as his own control in the evaluation of short-term effects of surfactant therapy. Serial observations (ABGs, cardiac output, lung compliance and chest radiographs) will

be made to establish a baseline period of 6 hrs. A randomized crossover study of surfactant vs. placebo treatment will then be done. Patients will receive treatment A and B in random order separated by 3 hours. Treatment definitions are: (A) Transbronchoscopic administration of 10 cc air into each of the 5 lobar bronchi followed by a 3 hr data-collection period; (B) Transbronchoscopic administration of 10 cc bovine surfactant into each lobar bronchus. Group I consists of those receiving the air first, and Group II consists of those receiving surfactant first. We have found that it is impossible to keep the bedside investigator blinded to the vial contents because of the bubbly appearance of surfactant.

Immediately prior to each instillation a BAL procedure will be done During the 3 hour data collection periods, the investigators will collect ABG's, lung volume and compliance data, pulmonary arterial pressures and cardiac output, chest radiographs, and bronchial secretions for phospholipid analysis as detailed below. Three hours after the second treatment, a third BAL will be performed.

Uncontrolled observations will be made of the longer term effects of surfactant, realizing that the natural history of ARDS in a given patient is unpredictable. Data on lung compliance, gas exchange and chest radiographs will be collected over a 1 week period. Endpoints examined will be length of ventilator dependence, signs of barotrauma and survival. Sequential BAL specimens will be obtained before and after therapy for biochemical analysis and blood will be obtained to assay for the development of systemic immunization to bovine surfactant.

c. Protocol of Data Collection:

(1) Pre-Treatment Phase (t = 0-6 hrs)

- Background information recorded and skin testing performed.
- Pulmonary capillary wedge pressure, cardiac output and PVR will be recorded.
- CXR, portable, supine and erect.
- ABG's will be at t = 0, 3, 6 hrs.
- Pneumotachograph is calibrated and placed in line for the Respitrace calibration.
- Respitrace inductance vests are taped in place and calibrated. Continuous recording begun at t = 4 hrs (continued for 8 hrs).
- Plateau airway pressures is recorded as a mean of 3 values.
- Pneumotachographic record of exhaled volume is made for each volume change.
- Random allocation to Group I or II is done at this time.

(2) First Treatment Phase (t = 6 hrs)

- Five syringes containing 10 cc each of either porcine surfactant (at 80 mg/cc) or air

will be drawn.

- A BAL will be performed.

- 10 cc from the assigned syringe (0.8 gm surfactant or air) followed by a 1 cc air flush is infused via the bronchoscope channel directly into one of the five lobar bronchi. Fifteen minutes later, the contents of the remaining 4 syringes will be infused into each of the remaining four lobar bronchi.

(3) First Post-Treatment Phase (t = 6-9 hrs.)

- ABG's will be obtained at t = 5', 15', 30', 60', 2 hr., 3 hr. after instillation. FIO_2 will not be adjusted during this period unless required for patient hypoxemia.
- Airway pressures and ventilator parameters will be recorded at 5', 15', 30', 1 hr, 2 hr, 3 hr after instillation.
- Bronchoscopy will be repeated at 3 hours after instillation for BAL specimens for determination of elastase and, alpha-1-PI levels and activities albumin, and phospholipid analyses. The second treatment phase will be initiated during this bronchoscopy.
- Swan-Ganz measurements of PCWP, cardiac output and PVR will be done at 15', 1, 2, 3 hrs after instillation.

- CXR's will be obtained at 3 hrs after instillation.

(4) Second Treatment Phase (t = 9 hrs)

- Identical to first treatment phase, except using the alternate syringes,and lavaging a previously unlavaged lobe.

(5) Second Post-Treatment Phase (t = 9 hrs)

- Identical to first post-treatment phase. The second post treatment phase will end at 12 hours with bronchoscopy and lavage of a previously unlavaged lobe with 50 ml saline only if surfactant was given in the second treatment phase.

(6) Long-Range Data Collections

- CXR, as above, daily until ICU discharge.
- Swan-Ganz data as above at t = 24, 48 hrs.
- Repeat bronchoscopy with BAL at t = 24 hrs.
- Assay will be done for circulating antibody and immune complexes to surfactant on blood samples collected on days 0, 30 and at at 6 months.

2. Bronchoalveolar lavage protocol: A fiberoptic bronchoscope will be wedged into a right middle lobe or lingular bronchial subsegment and 60 cc 0.9% NaCl slowly infused; immediately thereafter gentle suction will be applied to remove as much of the infused volume as possible. This specimen will be centrifuged at 200 G for 10 min.; the cell pellet will be resuspended and examined for WBC count and differential, and the supernatant reserved at -20° C for subsequent assays.

3. Data analysis: Comparison of post-treatment parameters of gas exchange, lung volume, and inflammation to pre-treatment values and to placebo-treatment values is done using analysis of variance and covariance.

   Uncontrolled observations are made of the longer term effects of surfactant realizing that natural history of ARDS in a given patient is unpredictable. Data on lung compliance, gas exchange, and chest radiographs is collected over a one week period. Endpoints examined are length of ventilator dependence, evidence of barotrauma, and survival. Sequential BAL specimens are obtained before and after therapy for biochemical analysis and blood is obtained to assay for the development of systemic immunization to bovine surfactant.

4. Experimental methods:

   a. Plasma analyses:

      (1) Assays of α_1-PI level and activity and were performed as described under section 6. A. 4. b.
      (2) Immunologic studies:

      Surfactant-Anti-Surfactant Immune Complexes (81): Rabbit antibody to human surfactant is used to coat plastic plates (Immulon, Dynatech, Inc.) at a concentration of 1 μg antibody per well in a 96 well microtiter plate. The antibody is prepared by immunizing rabbits with human surfactant, absorbing the antisera from these rabbits with normal human serum proteins, and preparing an ammonium sulfate precipitate of the rabbit serum x 2. After plating the IgG portion of the immune rabbit serum, the plates' protein binding capacity is blocked by coating the plates further with bovine serum albumin (BSA). Control plates are coated with BSA alone. The patient's serum is serially diluted and then added to the appropriate wells. The plates are incubated overnight at 4°C, washedwith notmal saline, and then incubated with alkaline phosphatase-conjugated rabbit-anti-human IgG. After overnight incubation, the plates are then washed, and alkaline phosphatase substrate (Sigma Chemical Co., St. Louis, MO) in the appropriate buffer is added. Time is allowed for the color to develop (30-40 min) and the A_{405} is measured on an automated ELISA reader, with the background absorbance (wells not receiving patient's sera) subtracted.

Background binding (A_{405} for plates coated with BSA) is subtracted from A_{405} for corresponding wells in plates coated with surfactant antibody. An A_{405} value of ≥ 0.1 is taken as significant.

Anti-Surfactant Antibody (82): This assay is performed identically to that for immune complexes, with the following exception: The wells in the test plates are coated with 500 ng/well human surfactant instead of anti-surfactant antibody. Therefore, this assay tests for the presence of free anti-surfactant antibody, rather than immune complexes between surfactant and anti-surfactant.

b. Bronchoalveolar lavage analyses:

(1) Assays of cell number and differential, α_1-PI level and activity, α_1-PI-HNE complex level, HNE activity and albumin concentration were performed as described in section 6. A. 4. c.

(2) Phospholipid concentration: BAL samples used are from the supernatant obtained after a first centrifugation (250 x g), to remove cells. Lipids are extracted from a 0.3 - 0.6 ml aliquot of each BAL according to the Bligh & Dyer method (83): The BAL sample is diluted to 0.8 ml with 0.9% NaCl. After addition of 2 ml of methanol and 1 ml of chloroform, the mixture is vigorously agitated (vortex). To separate two phases, 1 ml of chloroform and 1 ml of 0.9% NaCl are added and the samples are centrifuged at 250 x g for 20 minutes. The lower phase is aspirated and transfered in a pyrex tube out of which the solvent is evaporated under a stream of air.

Phorsphorus assay (84): Residual solvent is removed by placing pyrex tubes in an oven at 140°C for 1 hour. Acid hydrolysis of samples is done by adding 0.3 ml of 70% perchloric acid to dry samples and heating at 180°C for 20 min in a heating block using a reflux condensers. Reagents are added in the following order (mixing after each addition): 1.0 ml distilled water, 0.4 ml 1.25% ammonium molybdate, 0.4 ml 5% ascorbic acid. The samples are then heated for 5 min in a boiling water bath. When cool, they are centrifuged at 250 x g for 5 min and absorbance is read at 797 nm. A standard curve is made with known amount of KH_2PO_4 from 0.04 μg to 8 μg. Conversion from phosphorus to phospholipid is made using the following formula: (μg P)/(30.97) = μmol P x 0.857 = mg dry phospholipids.

(3) Phospholipid profile by thin layer chromatography: After extraction of lipids from the sample (as described above), 250-500 μg phospholipid are concentrated in 10 μl chloroform. Silica plates (10 x 10 cm HPTLC plates silica gel 60 (E. Merck, Darmstadt, Germany) are sprayed with 0.4 M boric acid and then activated in an oven at 120°C for 60 min. When

plates are at room temperature, the sample is applied at the right lower corner of the plate, 1.5 cm from edges. The plates are developed 7 cm in the first dimension with chloroform/methanol/water/ammonia (70:30:3:2). The plates are then dried for 30 min in a container flushed with nitrogen. The plates are developed 7 cm in the second dimension with chloroform/methanol/water (65:35:5). Lipids are revealed with iodine vapors and identified relative to phospholipid standards. A photocopy of the plate is taken and spots are scraped. Silica is transferred into pyrex tubes for phosphorus determination.

(4) Surfactant Function: Sample preparation: After phosphorus determination for each sample, a known amount of phospholipid is transferred into dialysis bags. Samples are dialyzed against water for 24 hours at 4°C. Dialyzed samples are then lyophilized. To each dry sample, 0.1 μCi of ^{3}H-DPPC is added and samples are resuspended by vortexing in 0.6% NaCl 1.5 mM $CaCl_2$ at a phospholipid concentration of 2 mg/ml. One third of each sample is saved and labeled "Plain". The remaining volume is extracted (Bligh & Dyer). After evaporation of chloroform, lipids are resuspended in 0.6% NaCl 1.5 mM $CaCl_2$ at a calculated phospholipid concentration higher than 2 mg/ml and labeled "Extracted". Radioactivity in 2 μl of each "Plain" and "Extracted" samples is determined. Based on cpm, "Plain" and "Extracted" samples from the same BAL are precisely equilized at a phospholipid concentration of 2 mg/ml in 0.6% NaCl 1.5 mM $CaCl_2$.

Determination of surface tension activity: Samples to be analyzed are kept on ice. 35 μl are loaded into a plastic chamber, placed immediately in the Pulsating Bubble Surfactometer (85) at 37°C. A bubble is created (small diameter: 0.4 mm) and after 15 seconds, pulsation is started at 20 cycles per min for 5 minutes. Values for minimum surface tension are read from the printed output.

(5) Surfactant pool size (^{14}C-DPPC dilution): Samples of crude BAL or of extracted BAL phospholipid are counted for ^{3}H content.

c. Surfactant: Surfactant is isolated from minced porcine lungs by chloroform-methanol extraction and liquid-gel chromatography. The latter procedure separates the original surfactant material into phospholipids, cholesterol, triglycerides, and cholesteryl esters. Only the first of these fractions, which in addition to phospholipids contains about 1% proteins with a high proportion of hydrophobic amino acids, is used in the present trial. The composition of this material is shown:

Phospholipids	Percent Total Phospholipid
Phosphatidylcholine	79.9[a]
Phosphatidylethanolamine	6.9
Phosphatidylserine	1.3
Phosphatidylinositol	5.2
Phosphatidylglycerol	1.2
Lysophosphatidylcholine	-
Sphingomyelin	5.5
Other phospholipids	-

[a] Dipalmitoylphosphatidylcholine constitutes about 33% of this fraction

The physiological activity of this surfactant has been documented with pulsating bubble and in experiments on artificially ventilated preterm newborn rabbits. It has recently shown efficacy in a multi-center European trial of surfactant therapy of neonates with IRDS.

d. Skin Testing: Intradermal testing with 0.02 cc of the surfactant solution diluted 1:10, 1:100, or undiluted, is performed, and the reaction is measured (weal and flare) at 20 min. Negative control is saline; positive control is histamine (1 mg/ml) at 1:1000 or less dilute, or, when antihistamines or histamine receptor antagonists are present, codeine (30 mg/ml) undiluted.

Results in 10 normals show a non-specific reaction, with both weal and flare < 5 mm at 1:100. In these normals there is no associated lymphocyte proliferation response or release of IL-2 in response to surfactant (1 to 100 mg/ml; 5×10^5 lymphocyte/ml, 3-6 days in culture). Surfactant does not inhibit the positive control proliferative response to PHA or Con-A. Thus, we are comfortable in believing the small weal and flare seen in normals is a non-specific response to the intra-dermal injection of lipid.

Patients are excluded from this study: (a) if the intradermal test is > 5 mm (weal or flare) for the 1:10 dilution or > 2 mm for the 1:100 dilution; or (b) if there is no reaction to the positive control).

e. Lung Mechanics: Changes in measured total thoracic compliance will be assumed to represent the changes in lung compliance over a brief timespan (8 hours). The assumption is made that chest wall compliance will not change over this time period. Static thoracic compliance is defined by the formula P_{mouth}/ V. Plateau pressure recorded by the line- pressure manometer of the ventilator (by dialing in an inspiratory pause) and is used to measure Pmouth.

2. Results

a. Patient enrollment: During the course of this study, it was possible to enroll and analyze samples from a total of six patients fitting study criteria. A much larger number of patients were evaluated for study, but were not able to be enrolled. Enrollment was prevented by a variety of circumstances. These included: inability to obtain informed consent, presence of underlying lung disease; presence of ARDS more than 48 hrs prior to evaluation; presence of active pneumonia or HIV infection; and moribund status with expectation of imminent death.

Brief summary of the medical history of each enrolled patient:

#1 RB was a 30 year old white male with a history of diabetes since age three, coronary artery disease with myocardial infarction, and chronic renal failure. He presented with deep infection of his right foot and a gangrenous second toe. Open drainage and debridement was performed in the operating room, and the patient developed ARDS during the post-operative period and required continuing mechanical ventilation; a pneumothorax required insertion of a chest tube. Surfactant was administered as per protocol without complication. No further evidence of barotrauma was seen. The patient's requirement for mechanical ventilation persisted for 4 days. He was discharged alive from the hospital.

#2 CS was a 33 year old white female with a history of chronic alcoholism who presented with abdominal pain and had laboratory findings consistent with acute pancreatitis. On the fourth hospital day she was found to have developed ARDS, hypophosphatemia, and acute renal failure. Surfactant was administered per protocol on the fifth hospital day without complication. The patient's requirement for mechanical ventilation persisted for 38 days; she was successfully weaned and discharged from the ICU. No evidence of barotrauma was seen. She was subsequently found pulseless on the hospital ward and could not be resuscitated.

#3 RV was a 61 year old hispanic male with a history of chronic alcoholism and seizure disorder who had a witnessed seizure that resulted in admission to the Neurology service. He developed respiratory failure considered secondary to aspiration, and on the sixth hospital day required intubation and satisfied criteria for ARDS. Surfactant was administered that same day per protocol on the fifth hospital day without complication. Septicemia was documented by growth of acinetobacter and flavobacterium from the patient's blood. He responded to antibiotic therapy, and mechanical ventilation was discontinued 10 days after surfactant treatment. No evidence of barotrauma was seen. The

patient was discharged from the hospital alive.

#4 RR was a 26 year old hispanic female involved in a motor vehicle accident. She received emergency laparotomy with splenectomy and a left chest tube. She developed ARDS on the 8th hospital day, and surfactant was administered without complication. Her course was complicated by intracranial hemorrhage and unremitting hypotension, and she expired on the 14th hospital day.

#5 JD was a 28 year old hispanic male with a history of paraplegia secondary to a gunshot wound. Hospital admission was due to urosepsis accompanied by hypotension. Despite antibiotic therapy he developed bilateral renal abscesses. ARDS developed on the 4th hospital day, and surfactant was administered per protocol without complication. The patient gradually recovered pulmonary and renal function, and mechanical ventilation was discontinued 33 days after surfactant treatment. The patient was discharged on the 47th hospital day.

#6 JA was a 46 year old Iranian male with severe chronic alcoholism with a past history of hemorrhagic pancreatitis and glucose intolerance. He was admitted to hospital because of abdominal pain and had laboratory findings consistent with acute pancreatitis. Respiratory failure requiring intubation and mechanical ventilation developed on the 3rd hospital day, and on the 4th hospital day he received surfactant per protocol without complication. Pulmonary function recovered slowly, and he was weaned from the ventilator on the 34th hospital day. He was discharged from the hospital alive.

A summary of patient characteristics is presented in Table 17.

b. Clinical observations

During the period of the experimental protocol there were no significant changes in cardiac output or hemoglobin content, and, consistent with this, no changes in venous saturation. In addition, the inspired oxygen concentration was not altered during the course of treatment of each patient. Thus, alterations in gas exchange are accurately reflected in alterations in the arterial oxygen tension.

Figure 12A displays alterations in p_aO_2 that occurred in the three patients who received placebo prior to receiving surfactant. A modest decrement in gas exchange is demonstrated in two cases (#4 and #5), coincident with insertion of the fiberoptic bronchoscope into the airway. After receiving surfactant, one patient (#5) sustained an increase in p_aO_2 that was sustained

for several hours. Figure 12B displays alterations in p_aO_2 that occurred in the three patients who received surfactant prior to receiving placebo. Two patients, (#3 and #6) sustained a modest and brief increase in p_aO_2 after receiving surfactant, while Pt # 2 had a more marked increase in p_aO_2 that was sustained for more than 24 hours. A modest decrement in gas exchange occurred in two patients (#2 and #3) after placebo, while Pt #6 had no change. Table 18 summarizes the difference in p_aO_2 seen just before treatment with surfactant or placebo and the greatest value of p_aO_2 measured within 30 minutes after treatment.

The average change in p_aO_2 with surfactant was +26.5 torr (rang[illegible]o 42) and occurred in 4 patients five minutes after treatment and in two patients 30 minutes after treatment. This change was significantly different ($p = 0.04$, Mann Whitney U Test) from the change in p_aO_2 with placebo (-6 torr, range -27 to +26) that occurred in three patients five minutes after placebo and in three patients 30 minutes after placebo (Table 18).

Summary data pertaining to gas exchange, ventilation, and hemodynamics are shown for individual patients in Tables 19, 20, and 21. The change in effective compliance with surfactant and with placebo was slight, and not significantly different from the change seen with placebo (Figs. 13 and 14). Similarly, no significant change was seen in cardiac output (Figs. 15 and 16) or pulmonary artery pressure (Figs. 17 and 18).

c. Plasma analyses

Immunologic studies: The data displayed in Tables 22 and 23 represent the absorbance values from the assays for surfactant-anti-surfactant immune complexes and for free anti-surfactant antibodies, respectively. Serum samples were serially diluted and assayed as described. Control determinations were made in which wells were coated with normal rabbit serum; absorbances under these conditions were ≤ 0.008 absorbance units (data not shown). Under conditions of these assays, an absorbance value of ≥ 0.1 is significant. Thus, the baseline sample and 2 week sample from patient #3 are suggestive of the presence of free anti-surfactant antibody, but there is no indication in any patient treated that either free antibody or immune complexes developed during followup observation. Fortunately, the followup observations span the 1 week to several month period during which an immune response might occur. The indigent nature of the patients treated prohibited more precise followup.

d. Bronchoalveolar lavage analyses

Cell count and differential: Analyses of cells recovered in lavage fluids obtained prior to and after surfactant administration are shown in Table 24. No significant alteration in cell yield or differential was found, consistent with the administered surfactant having little or no pro-

inflammatory activity. Many of the lavage fluids did demonstrate a marked preponderance of inflammatory cells, as previously reported in the lavage fluid of patients with ARDS

α_1-PI level: Levels of BAL α_1-PI, expressed relative to total BAL protein, changed very little during the periods of observation (see Table 25). This finding is consistent with the non-selective exudation of plasma proteins into the alveolar compartment of patient with ARDS.

α_1-PI activity: α_1-PI activity is interpreted most easily when expressed as specific activity; normal values are ≥ 0.80. At various times, abnormally low values were seen. These did not consistently coincide with any phase of surfactant treatment. The results indicate that α_1-PI specific activity was not affected by administration of exogenous surfactant as delivered in this protocol (see Table 25).

α_1-PI-HNE complex level: Levels of α_1-PI-HNE complex were recorded in all patients, consistent with the presence of an active inflammatory process in the lung. Levels of complex fell over the treatment course in one patient, but remained unchanged in the other 3 in whom adequate sample was available for analysis. Thus, these results also do not suggest any alteration in the inflammatory process resulting from exogenous surfactant as delivered in this protocol (see Table 25).

HNE activity: HNE activity, as measured by hydrolysis of synthetic substrate, was unmeasurable in the BAL fluids recovered from study patients. This result is consistent with the presence of active α_1-PI in each of the fluids analyzed (see Table 25).

Bronchoalveolar lavage surfactant: Analyses of bronchoalveolar lavage surfactant levels, composition, and function were hampered by the small volumes of lavage fluid used in this study, by the subjective nature of the analysis of phospholipid composition, and by the amounts of phospholipid required for the analysis of composition. Nevertheless, certain observations are possible. In all but patient #4, instillation of exogenous surfactant was followed by recovery of increased amounts of phospholipid in BAL three hours later. Analysis of the lavage fluid from patient #4 obtained after instillation of surfactant was technically unsatisfactory, in that the aspirated fluid contained no detectable phospholipid. Whether this was due to problems with the procedure or with the laboratory analysis is unclear. In all other cases, lavage recovery of phospholipid increased up to ten times (see Table 26). Thus, instilled phospholipid of exogenous surfactant remains in the airways for at least three hours. In patients #1 and #5 where fluids from 1 day later were available for analysis, levels had fallen, consistent with removal of surfactant phospholipid from airways sampled by the lavage procedure.

Phospholipid profile (TLC) could be determined on six fluids that were recovered by lavage. The determination, done by thin layer chromatography, requires identification of

phospholipids that are revealed by iodine vapor staining (Figures 19, 20, 21) scraping of spots (where subjective decisions must be made regarding the boundaries between adjacent spots), and determination of phosphorus content of the scraped silica. Results (Table 27) are presented as percent total phospholipid. Profiles suggest some decrement in the fractional content of phosphatidylcholine, consistent with previously published reports. In all but the 1 day lavage in patient #1 and the lavage after treatment #1 in patient #6, these changes were mild; the two fluids cited showed marked depression of the PC content suggesting either gross abnormality of lung surfactant or contamination of the fluid with cell phospholipids. The latter may be more likely, since fluids collected at other times showed the expected profiles. The most significant finding associated with these determinations is that the quantity of lavage fluid used, and the amount of surfactant phospholipids recovered, will need to be increased if adequate determinations are to be obtained in future studies.

Surfactant function: Because we wished to determine if surfactant function was inhibited in patients with ARDS, and if this inhibition could be overcome by delivery of exogenous surfactant, determination of the function of BAL surfactant was made the highest priority. In all cases, the amount of phospholipid available for analyses was limited; adequate functional assays were performed before using fluids for other purposes. The results of these assays are shown in Table 28. Patients #1, #4, and #5 received placebo as treatment #1; patients #2, #3, and #6 received surfactant as treatment #1. In all but one case (patient #5) the greatest difference between non-extracted and extracted surfactant existed in the baseline lavage fluid, consistent with the presence of non-extractable inhibitor. In some (patients #1,2,3), this difference was fully overcome by the administration of exogenous surfactant. The one patient in whom it was possible to document that inhibitor function was overcome for 24 hours (#2) is the same patient who demonstrated prolonged improvement in gas exchange (Figure 4). In summary, the results demonstrate that, at least in individual patients, it is possible to overcome the inhibitor function present in lavage fluid.

Surfactant pool size (^{14}C-DPPC dilution): Due to the scant amount of phospholipid recovered in the lavage from the first patients studied, there were no counts recovered when ^{14}C-DPPC was instilled with the administered exogenous surfactant. For this reasons, radioactivity was not administered to additional patients.

3. Discussion: The studies described above provide the first view of the effect that exogenous surfactant may have in the treatment of patients with the adult respiratory distress syndrome. The rationale for using exogenous surfactant has been presented in the introduction to this report. Because the consequences of administration of surfactant were unknown, and because the safety of bronchoalveolar lavage was also not known at the time this study was designed, relatively small

quantities of surfactant were proposed, and relatively small volume lavages were performed. The results of this study have been presented in a variety of settings, and are being prepared for publication. They have had significant impact on the research that is currently being done on surfactant replacement in the setting of ARDS. Two prospective randomized multicenter studies of surfactant replacement are currently in progress, one sponsored by Ross-Abbott and the other by Burrows Wellcome. The former is closely related to the present study, in that surfactant derived from animal lungs is instilled into the endotracheal tube. In contrast to the study reported on here, and as a consequence of this study, the Ross-Abbott protocol provided, initially, for the delivery of a dose of 100 mg/kg, and this dose could be repeated up to 8 times. Subsequently, the dose has been decreased to 50 mg/kg. Thus, current views of adequate dosing include instillation of a total of 8 to 16 times the total amount of surfactant that was used in the current study. The protocol under investigation by Burrows Wellcome provides for aerosol delivery of Exosurf, a synthetic preparation that does not contain apoprotein. The amount delivered is difficult to ascertain, and results have, as yet, shown no benefit (E. Pattishall, M.D. - presentation at Roswell Park Symposium, March 27, 1991).

What aspects of the current study have provided encouragement to the pharmaceutical industry to pursue the studies cited above? First, there is a suggestion from the current study that administration of a single relatively small dose of exogenous surfactant results in improved gas exchange (Table 18). The effect was transient in five of the six patients studied and of modest magnitude. Nevertheless, it suggests that repetitive dosing may be of benefit, or that alternative methods of delivery may provide more significant benefit than seen in this study. However, for this pilot study, the results are clearly encouraging.

Secondly, administration of exogenous surfactant appears to, in certain cases, overcome the inhibitor function present in lavage fluid of patients with ARDS. The repetitive demonstration of this inhibitor function and the ability to overcome it are a novel and important result of the current study.

Finally, we have demonstrated the safety of administering relatively large quantities of surfactant (approximately 50 ml) to patients who are acutely ill with ARDS. We find that both physiological parameters of gas exchange and biochemical markers of inflammation suggest that no untoward reactions occurs.

This pilot study of surfactant replacement includes six patients; original plans called for study of eight patients. A large number of patients were reviewed for inclusion in the study, and two factor were responsible for a significant number of cases in which entry was not possible. These were the desire to study patients early in the course of their disease and the requirement that informed consent be obtained from the patient. The former requirement continues to be desirable, and our experience with this study has suggested that it is not practical to require enrollment of patients in

less than 48 hours after the time of diagnosis of ARDS. The latter requirement is not practical, and studies of acute illness will be performed with greater facility if it is altered.

7. CONCLUSIONS: Two clinical pilot studies were accomplished under support of the current contract.

The first study focused on the administration of α_1-PI to patients with the adult respiratory distress syndrome and had the goals of determining safety, describing pharmacokinetic parameters, and determining whether intravenous administration of α_1-PI would augment functional antiprotease activity of blood and/or bronchoalveolar lavage fluid. Conclusions reached from this study include the following:

1. Administration of α_1-PI to patients with ARDS was not accompanied by evidence of toxicity. No alterations in vital signs occurred during or immediately after the infusion; further, no alterations in hematologic, renal, hepatic or other clinical parameters could be attributed to administration of α_1-PI.

2. The central volume of distribution for α_1-PI in patients with ARDS was not different from that measured in control (emphysema) patients. This finding is not unexpected since the central volume of distribution of α_1-PI is probably identical to the plasma volume. Pharmacokinetic analyses demonstrated substantial alterations in the behavior of α_1-PI. The movement of α_1-PI out of the central volume of distribution occurred with a half life 50% shorter than that of a control patient population. This shortened transit time is likely to be a reflection of enhanced permeability for α_1-PI across the endothelium. The steady state clearance of α_1-PI from the central compartment was also decreased to 60% of control values. The change in both half times and the lack of change in the volumes of distribution for α_1-PI lead to a calculated value for the clearance of α_1-PI in ARDS of 0.816 ± 0.057 ml kg^{-1} hr^{-1}; a clearance that is one third that found in control patients. These data suggest that metabolism of α_1-PI is substantially enhanced in ARDS and they have important implications for dosing intervals during the therapeutic administration of α_1-PI.

3. Basal plasma levels of α_1-PI, an acute phase reactant, were substantially elevated. The infusion of 60 mg/kg of plasma-derived α_1-PI resulted in a mean increment α_1-PI plasma levels of 1.24 ± 0.09 mg. There was a corresponding increment in the levels of plasma anti-elastase activity, demonstrating that the infused α_1-PI retained all of its functional activity. The steady state T½ of 37.9 hours and the volume of distribution measured using data derived from the antigenic assays are in close agreement with those calculated using plasma radioactivity and suggest that the radiolabeled α_1-PI and the infused α_1-PI behaved similarly.

4. There was no measurable increase in the BAL levels of α_1-PI after the infusion, despite the fact that increased BAL radioactivity levels demonstrated passage of the infused α_1-PI into the lung. Basal BAL levels of α_1-PI were elevated and the infused α_1-PI only augmented plasma levels by 28%. The calculated level of infused α_1-PI in the BAL suggests that plasma and BAL had largely reached equilibrium within 6 hours of infusion, and thus administration of α_1-PI directly into the alveolar space as an aerosol will also require a significantly reduced dosing interval in order to maintain therapeutic α_1-PI levels.

5. The functional activity of the lavage α_1-PI did not change after the infusion. Since the calculated contribution of the infused α_1-PI to those levels is significant, this observation indicates that α_1-PI that reached the alveolar space retained activity.

6. Results of this study support a therapeutic approach aimed at providing consistently elevated lung α_1-PI levels of sufficient magnitude that any protease released locally can be inhibited. If the release of protease is a local phenomenon, then augmentation of α_1-PI might be optimally performed by local administration as well. Technology exists for aerosol administration of α_1-PI and our data support such use in ARDS.

The second pilot study that was performed focused on the possible benefits to gas exchange and lung compliance that might accompany administration of exogenous lung surfactant to patients within the first 48 hours of development of ARDS. Conclusions drawn from this study include the following:

1 Our observations suggest that administration of a single relatively small dose of exogenous surfactant results in improved gas exchange. Repetitive dosing may be of benefit, and alternative methods of delivery may provide more significant benefit than seen in this study. However, for this pilot study, the results are clearly encouraging.

2. Administration of exogenous surfactant appears to, in certain cases, overcome the inhibitor function present in lavage fluid of patients with ARDS. The repetitive demonstration of this inhibitor function and the ability to overcome it are a novel and important result of the current study.

3. Administration of relatively large quantities of surfactant (approximately 50 ml) to patients who are acutely ill with ARDS was tolerated well. We found that both physiological parameters of gas exchange and biochemical markers of inflammation suggested no untoward reaction.

4. No alterations in lung compliance or chest radiograph were found following the single 50 mg/kg dose of surfactant administered to patients in this study.

5. Neither immune complexes containing anti-surfactant antibody nor free anti-surfactant antibody were detected in the plasma of patients receiving surfactant.

6. Further research is required to identify the optimal dosing strategy to employ in delivery of exogenous surfactant to patient with ARDS.

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APPENDIX I: TABLES

TABLE 1

Surfactant abnormalities in Patients with ARDS[+]

INVESTIGATOR	YEAR	NUMBER OF PATIENTS	TOTAL PL	PC OR DPPC	PG	MINIMUM SURFACE TENSION
Ashbaugh{{1418}}	1967	2				I
Petty {{1850}}	1977	1	NC			NC
Petty {{1870}}	1979	6				NC
Hallman {{855}}	1982	26	NC	D	D	I
Pison {{1424}}	1989	17	D	D	D	NC
Pison {{1871}}	1990	30	D	D	D	

[+] NC = No change; I = Increased; D = Decreased; PL = Phospholipid; PC = Phosphatidylcholine; DPPC = Disaturated phosphatidyl choline

TABLE 2

Published Controlled Treatment Studies of
Exogenous Surfactant in Human Infants

Investigator	Year	Surfactant composition	Dose	Outcome*
Hallman{{1802}}	1983	Human amniotic fluid surfactant	70 μmol lipid phosphorus (60 mg dry surfactant) /kg	IV
Merritt {{1812}}	1983	Human amniotic fluid surfactant	60 mg surfactant/kg	IV
Wilk ·.on{{1868}}	1985	Synthetic: DPPC 70%, PG 30%	25 mg	IV, DM/PBD
Hallman {{1801}}	1985	Human amniotic fluid surfactant	60 mg/kg	IV, NDM DM/PBD
Fujiwara {{1866}}	1987	Surfactant TA (bovine)	100 mg/kg	IV,NDM, DM/PBD
Gitlin {{1862}}	1987	Surfactant TA (bovine)	100 mg phospholipid/kg	IV,NDM, NDM/PBD
Raju {{1863}}	1987	Surfactant TA (bovine)	100 mg/kg	IV, NDM, DM/PBD
Svenningsen{{1864}}	1987	Curosurf (porcine)	200 mg/kg	IV
Collaborative European Multicenter Group {{117}}	1988	Curosurf (porcine)	200 mg/kg	IV, DM
Horbar {{1869}}	1988	Surfactant TA(bovine)	100 mg/kg	IV,NDM, NDM/PBD

* IV = improved ventilation; DM = decreased mortality; NDM = no decreased mortality; DM/PBD = Decreased mortality or incidence of bronchopulmonary dysplasia; NDM/PBD = no DM/BPD

TABLE 3

Published Controlled Prevention Studies of
Exogenous Surfactant in Human Infants

Investigator	Year	Surfactant composition	Dose	Outcome[+]
Morley {{1793}}	1981	Synthetic: DPPC 70%, PG 30 %	25 mg	NIV
Milner {{1815}}	1983	Synthetic: DPPC 70% PG 30 %	25 mg x 2	NIV
Halliday {{1813}}	1985	Synthetic: DPPC 70% PG 30 %	3 - 5 ml	NIV, NDM
Enhorning {{1309}}	1984	Calf lung surfactant extract (CLSE)	75-100 mg PL	IV, DM
Shapiro {{tim27}} Kendig {{1867}}	1985	CLSE	100 mg PL	?IV, NDM
Kwong {{1860}}	1988	CLSE	100 mg PL	IV, NDM
Wilkinson {{1861}}	1985	Synthetic: DPPC 70%, PG 30%	25 mg	NIV, NDM
Merritt {{1846}}	1985	Human amniotic fluid	60 mg at birth	IV, DM/PBD
Ten Center Study Group	1986 1987	Synthetic: DPPC 70%, PG 30%	100 mg	DM

[+] IV = improved ventilation; NIV = No improved ventilation; DM = decreased mortality; NDM = no decreased mortality; DM/PBD = Decreased mortality or incidence of bronchopulmonary dysplasia; PL = Phospholipid.

TABLE 4

Characteristics of Patients Receiving α_1PI

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Etiology of ARDS	Sepsis	Pneumonia Sepsis	Sepsis	Sepsis	Sepsis/amniotic fluid embolus	Multiple transfusions	Sepsis
Age (yrs)	64	48	39	44	27	37	29
Weight (kg)	78	75	51.4	62.9	66.6	45.0	81.4
Days on ventilator	14	29	10	63	8	10	24
Static lung compliance (ml/cm H_2O)	11.8	12	16.0	11.4	14.0	22.7	10.8
P_aO_2 (mm Hg)	70	82	113	108	68	104	64
f_iO_2	0.45	0.45	0.45	0.4	0.65	0.5	0.55
P_aO_2/f_iO_2	155.56	182.22	251.11	270.00	104.62	208.00	116.36
PEEP (cm H_2O)	5.0	5.0	5.0	5.0	10.0	0.0	5.0

The physiologic characteristics for each patient at the time of study enrollment are shown in this table.

TABLE 5

Hematologic Values with Infusion of α_1PI

Parameter	Preinfusion Mean ± SEM	24 hours Mean ± SEM
Hemoglobin (mg/dl)	9.9 ± 0.6	10.2 ± 0.4
Hematocrit (%)	30.0 ± 1.6	31.0 ± 1.1
Platelets (1000/μl)	219 ± 32	168 ± 32
White cells (1000/μl)	15.6 ± 1.8	15.4 ± 1.7
Differential (% of total)		
PMN	61 ± 4.0	67 ± 4.0
Band forms	10 ± 1.0	7 ± 2.0
Lymphocytes	19 ± 4.0	16 ± 3.0
Monocytes	5 ± 1.0	5 ± 1.0
Eosinophils	3 ± 1.0	4 ± 3.0

A complete blood count, platelet count, and cell differential was performed on blood samples obtained during the baseline observation period, and at one day after the administration of the α_1-PI. No significant changes occurred in any of the measured parameters when post-infusion values were compared to those at baseline. Data from each time point are presented here as mean ± SEM.

TABLE 6

Serum Chemistry Values with Infusion of α_1PI

Parameter	Pre-infusion Mean ± SEM	Day four Mean ± SEM
BUN (mg/dl)	42.9 ± 15.1	29.7 ± 7.3
Creatine (mg/dl)	1.1 ± 0.4	0.9 ± 0.2
Bilirubin (mg/dl)	1.9 ± 0.7	1.9 ± 0.7
SGOT (IU/l)	57.9 ± 8.2	54.7 ± 10.2
SGPT (IU/l)	25.6 ± 6.3	13.9 ± 4.6
LDH (IU/l)	232.7 ± 97.6	273.0 ± 126.0
Alkaline phosphatase (mg/dl)	189.1 ± 17.8	223.1 ± 36.8
Serum protein (g/dl)	5.8 ± 0.4	5.9 ± 0.4

A serum chemistry panel was performed on blood samples obtained during the baseline observation period, and at 4 days after the administration of the α_1-PI. No significant changes occurred in any of the measured parameters when post-infusion values were compared to those obtained at baseline. Parameters relating to renal, hepatic, and metabolic function for each time point are summarized in this table. Data are presented as mean ± SEM.

TABLE 7

Results of Plasma α_1-PI Analyses from Individual Patients

TABLE 7 (cont'd): Patient 1

Plasma α_1PI Analyses

Time (hours)	Antigenic mg/ml	Activity mg/ml	Complex ng/ml
0.00	2.78	2.90	611.1
0.25	4.12	3.69	631.1
0.50	4.26	4.18	531.1
0.75	3.98	4.24	539.7
1.00	4.04	4.63	483.2
1.50	3.75	4.44	484.4
2.00	3.76	4.29	537.6
2.50	4.10	3.68	651.1
3.00	3.96	3.86	541.2
3.50	4.01	4.38	675.1
4.00	4.26	3.92	473.6
4.50	4.02	3.92	472.3
5.00	3.93	4.11	494.4
5.50	3.67	3.71	576.3
6.00	4.50	3.82	494.9
8.00	4.35	3.62	502.4
16.00	3.68	4.22	479.0
24.00	3.98	4.41	873.7
48.00	3.60	4.16	602.7
72.00	3.28	3.45	1352.0
96.00	3.59	3.85	2219.0
120.00	4.12	4.20	602.0
168.00	3.79	4.23	628.3

TABLE 7 (cont'd): Patient 2

Plasma α_1PI Analyses

Time (hours)	Antigenic mg/ml	Activity mg/ml	Complex ng/ml
0.00	3.85	3.25	320.8
0.25	5.20	5.18	251.6
0.50	5.22	5.04	260.1
0.75	5.85	6.36	304.4
1.00	5.35	5.47	301.1
1.50	4.86	5.82	295.0
2.00	5.02	3.27	284.2
2.50	4.06	4.83	268.2
3.00	4.42	4.06	254.3
3.50	4.87	3.79	273.4
4.00	4.44	4.67	314.8
4.50	5.11	4.13	285.7
5.00	4.51	5.15	229.8
5.50	4.40	3.44	272.6
6.00	4.54	5.46	323.0
8.00	3.99	5.50	404.0
16.00	4.23	5.46	526.5
24.00	3.82	5.25	445.1
48.00	3.99	5.15	366.9
72.00	3.78	4.52	301.7
96.00	3.39	3.58	307.4
120.00	3.48	4.14	454.1
168.00	3.51	3.49	384.8

TABLE 7 (cont'd): Patient 3

Plasma α_1PI Analyses

Time (hours)	Antigenic mg/ml	Activity mg/ml	Complex ng/ml
0.00	4.84	4.52	798.6
0.25	6.29	6.09	560.9
0.50	6.20	5.33	218.9
0.75	6.37	5.67	383.4
1.00	6.74	5.90	304.2
1.50	6.93	5.79	269.7
2.00	6.37	6.22	250.5
2.50	7.11	5.96	233.8
3.00	6.56	5.98	238.9
3.50	6.65	6.36	258.6
4.00	6.57	5.16	254.7
4.50	6.46	5.05	246.2
5.00	6.74	5.77	212.7
5.50	6.29	5.50	212.4
6.00	6.37	4.61	211.2
8.00	6.02	4.74	379.7
16.00	5.84	4.57	276.4
24.00	5.49	4.69	201.0
48.00	4.84	4.64	245.0
72.00	4.76	4.01	232.0
96.00	4.52	4.23	250.0
120.00	4.44	4.18	251.4
168.00	4.68	5.29	226.5

TABLE 7 (cont'd): Patient 4

Plasma α_1PI Analyses

Time (hours)	Antigenic mg/ml	Activity mg/ml	Complex ng/ml
0.00	3.75	3.36	251.7
0.25	5.51	4.69	197.8
0.50	5.04	4.72	264.2
0.75	5.28	4.84	181.4
1.00	5.40	4.81	324.5
1.50	5.43	4.50	229.8
2.00	5.10	3.98	267.6
2.50	5.28	4.43	216.5
3.00	5.28	4.65	190.1
3.50	5.43	4.80	288.2
4.00	5.23	4.67	216.6
4.50	5.12	4.62	196.5
5.00	5.12	4.28	200.7
5.50	4.48	4.43	289.2
6.00	4.71	4.24	215.1
8.00	4.25	3.97	555.4
16.00	4.20	3.71	305.0
24.00	4.27	3.84	198.2
48.00			
72.00	3.96	3.64	172.1
96.00	3.89	3.64	207.8
120.00	3.89	3.71	157.0
168.00	4.01	3.89	125.3

TABLE 7 (cont'd): Patient 5

Plasma α_1PI Analyses

Time (hours)	Antigenic mg/ml	Activity mg/ml	Complex ng/ml
0.00	7.30	6.80	692.0
0.25	8.10	7.90	531.6
0.50	7.60	6.30	582.8
0.75	8.50		
1.00	8.50	7.70	613.4
1.50	9.10	7.80	989.8
2.00	8.20	7.40	620.8
2.50	8.00	7.30	760.2
3.00	7.90	7.80	615.2
3.50	8.10	7.70	536.2
4.00	7.70	7.60	630.2
4.50	8.10	7.70	880.8
5.00	8.40	7.00	711.4
5.50	7.30	7.10	612.8
6.00	9.76	8.80	560.8
8.00	8.42	7.30	864.1
16.00	7.55	7.30	783.9
24.00	7.55	7.04	413.8
48.00	7.72	7.82	592.3
72.00	8.05	8.05	451.2
96.00	7.72	7.20	335.6
120.00	5.32	5.00	373.1
168.00	4.17	4.00	592.4

TABLE 7 (cont'd): Patient 6

Plasma α_1PI Analyses

Time (hours)	Antigenic mg/ml	Activity mg/ml	Complex ng/ml
0.00	2.66	2.83	173.9
0.25	3.13	3.19	115.2
0.50	3.41	3.31	139.3
0.75	3.08	3.19	143.0
1.00	3.27	3.08	129.7
1.50	3.13	2.71	135.4
2.00	3.05	2.81	124.1
2.50	3.55	3.60	130.3
3.00	3.06	3.16	126.5
3.50	3.00	3.24	134.2
4.00	3.10	3.44	127.4
4.50	3.21	3.30	135.8
5.00	3.28	3.50	344.1
5.50	3.15	3.21	206.8
6.00	3.89	3.77	652.0
8.00	3.17	3.02	349.9
16.00	3.18	3.48	502.0
24.00	2.41	2.67	437.1
48.00	3.43	3.72	238.5
72.00	3.63	3.80	456.1
96.00	3.51	3.67	166.3
120.00	3.85	3.66	171.8
168.00	3.85	4.06	147.9

TABLE 7 (cont'd): Patient 7

Plasma α_1PI Analyses

Time (hours)	Antigenic mg/ml	Activity mg/ml	Complex ng/ml
0.00	5.13	4.78	375.5
0.25	6.57	6.27	331.6
0.50	5.56	6.10	307.7
0.75	6.57	6.73	359.1
1.00	6.72	6.66	310.4
1.50	6.36	6.36	381.1
2.00	6.00	5.91	320.3
2.50	6.50	5.70	354.5
3.00	5.85	6.29	343.4
3.50	5.71	3.19	315.8
4.00	6.50	5.93	372.4
4.50	6.00	6.32	369.5
5.00	6.14	6.39	370.0
5.50	6.43	6.10	366.2
6.00	6.42	6.35	361.2
8.00	7.23	6.16	251.0
16.00	6.53	5.97	326.9
24.00	6.58	6.45	244.1
48.00	6.09	6.42	372.5
72.00	6.53	6.02	426.8
96.00	5.22	5.42	391.3
120.00	6.58	6.07	252.4
168.00	5.88	4.82	163.6

TABLE 8

Plasma Radioactivities

TABLE 8

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Wt (kg)	68.4	75	51.4	62.9	66.6	45	81.4
A1AT Dose (mg)	4104	4500	3084	3774	3996	2700	4884
(CPM)	3.800E+08	2.882E+08	2.309E+08	1.293E+08	1.069E+08	6.746E+07	9.389E+07
	Plasma radioactivity (CPM/ml)						
Time (Hours)							
0	0	0	0	0	0	0	0
0.25	84900	78240	69067	46370	45123	21820	23870
0.5	81163	75870	62587	41810	36694	17432	20442
0.75	73561	74120	61213	40440	NA	14714	20283
1	67871	71030	56293	38690	32276	13170	20197
1.5	64163	64620	55600	36140	30278	11018	17476
2	61287	62660	53920	31430	28072	10101	15948
2.5	59095	58070	52453	29610	24992	9375	14932
3	55123	57350	48467	27110	24122	8733	14518
3.5	52300	56380	47747	26250	22521	8124	13728
4	52105	55280	45867	25800	21334	7734	13175
4.5	51531	55210	44853	23660	20310	7762	12105
5	50213	51340	42600	22470	19368	7069	12165
5.5	48724	48720	40893	20130	18295	6652	12110
6	46203	49480	39360	19830	17502	6393	11102
8	43596	46430	37213	NA	15180	5332	9973
14	NA	NA	NA	11270	NA	NA	NA
16	30177	36760	26533	NA	10075	3789	6772
22	NA	NA	NA	7930	NA	NA	NA
24	27853	29070	17147	NA	8534	2250	5618
30	NA	NA	NA	5690	NA	NA	NA
36	20180	NA	NA	NA	NA	NA	NA
48	16736	18030	9947	NA	5456	1802	3024
72	10393	11150	5680	NA	2838	1591	1608
73.75	NA	NA	NA	3160	NA	NA	NA
96	7468	7831	4560	NA	2021	1361	1040
101.75	NA	NA	NA	1552	NA	NA	NA
114	NA	NA	NA	1503	1014	NA	NA
120	5500	6096	2907	1261	NA	1380	885
144	NA	NA	NA	932	NA	1427	NA
168	3088	3525	2000	NA	905	NA	506
196	NA	NA	NA	545	NA	1163	NA
216	1777	2128	NA	NA	646	NA	NA
240	NA	NA	NA	344	555	1188	267
264	NA	NA	NA	NA	522	NA	NA
308	NA	NA	693	NA	NA	NA	NA

TABLE 9

Results of Plasma ^{125}I-α_1PI Analyses from Individual Patients

TABLE 9 (cont'd): Patient 1

Double Exponential Decay,
Statistical weighting

Variable	Value	Std. Err.
Initial value A	4.28192E+04	2.77305E+03
Rate alpha	2.45525E-01	3.16772E-02
Initial value B	3.88521E+04	2.33593E+03
Rate beta	1.65385E-02	1.01909E-03

	Time (hours)	CPM/ml	Calculated
1	2.50000E-01	8.49000E+04	7.89618E+04
2	5.00000E-01	8.11630E+04	7.64046E+04
3	7.50000E-01	7.35610E+04	7.39909E+04
4	1.00000E+00	6.78710E+04	7.17120E+04
5	1.50000E+00	6.41630E+04	6.75275E+04
6	2.00000E+00	6.12870E+04	6.37927E+04
7	2.50000E+00	5.90950E+04	6.04558E+04
8	3.00000E+00	5.51230E+04	5.74712E+04
9	3.50000E+00	5.23000E+04	5.47985E+04
10	4.00000E+00	5.21050E+04	5.24019E+04
11	4.50000E+00	5.15310E+04	5.02497E+04
12	5.00000E+00	5.02130E+04	4.83141E+04
13	5.50000E+00	4.87240E+04	4.65702E+04
14	6.00000E+00	4.62030E+04	4.49962E+04
15	8.00000E+00	4.35960E+04	4.00434E+04
16	1.60000E+01	3.01770E+04	3.06615E+04
17	2.40000E+01	2.78530E+04	2.62417E+04
18	3.60000E+01	2.01800E+04	2.14273E+04
19	4.80000E+01	1.67360E+04	1.75654E+04
20	7.20000E+01	1.03930E+04	1.18105E+04
21	9.60000E+01	7.46800E+03	7.94118E+03
22	1.20000E+02	5.50000E+03	5.33953E+03
23	1.68000E+02	3.08800E+03	2.41400E+03
24	2.16000E+02	1.77700E+03	1.09137E+03

TABLE 9 (cont'd): Patient 2

Double Exponential Decay,
Statistical weighting

Variable	Value	Std. Err.
Initial value A	3.50242E+04	2.79632E+03
Rate alpha	2.20522E-01	3.52887E-02
Initial value B	4.31645E+04	2.60633E+03
Rate beta	1.67101E-02	9.38565E-04

	Time (hours)	CPM/ml	Calculated
1	2.50000E-01	7.82400E+04	7.61301E+04
2	5.00000E-01	7.58700E+04	7.41731E+04
3	7.50000E-01	7.41200E+04	7.23121E+04
4	1.00000E+00	7.10300E+04	7.05421E+04
5	1.50000E+00	6.46200E+04	6.72561E+04
6	2.00000E+00	6.26600E+04	6.42791E+04
7	2.50000E+00	5.80700E+04	6.15793E+04
8	3.00000E+00	5.73500E+04	5.91280E+04
9	3.50000E+00	5.63800E+04	5.68995E+04
10	4.00000E+00	5.52800E+04	5.48708E+04
11	4.50000E+00	5.52100E+04	5.30214E+04
12	5.00000E+00	5.13400E+04	5.13328E+04
13	5.50000E+00	4.87200E+04	4.97885E+04
14	6.00000E+00	4.94800E+04	4.83736E+04
15	8.00000E+00	4.64300E+04	4.37639E+04
16	1.60000E+01	3.67600E+04	3.40660E+04
17	2.40000E+01	2.90700E+04	2.90800E+04
18	4.80000E+01	1.80300E+04	1.93556E+04
19	7.20000E+01	1.11500E+04	1.29603E+04
20	9.60000E+01	7.83100E+03	8.67851E+03
21	1.20000E+02	6.09600E+03	5.81132E+03
22	1.68000E+02	3.52500E+03	2.60576E+03
23	2.16000E+02	2.12800E+03	1.16840E+03

TABLE 9 (cont'd): Patient 3

Double Exponential Decay,
Statistical weighting

Variable	Value	Std. Err.
Initial value A	4.32926E+04	3.15057E+03
Rate alpha	1.13751E-01	1.57231E-02
Initial value B	2.06033E+04	3.29772E+03
Rate beta	1.56167E-02	1.91964E-03

	Time (hours)	CPM/ml	Calculated
1	2.50000E-01	6.90670E+04	6.26018E+04
2	5.00000E-01	6.25870E+04	6.13421E+04
3	7.50000E-01	6.12130E+04	6.01157E+04
4	1.00000E+00	5.62930E+04	5.89218E+04
5	1.50000E+00	5.56000E+04	5.66278E+04
6	2.00000E+00	5.39200E+04	5.44532E+04
7	2.50000E+00	5.24530E+04	5.23913E+04
8	3.00000E+00	4.84670E+04	5.04361E+04
9	3.50000E+00	4.77470E+04	4.85816E+04
10	4.00000E+00	4.58670E+04	4.68224E+04
11	4.50000E+00	4.48530E+04	4.51533E+04
12	5.00000E+00	4.26000E+04	4.35693E+04
13	5.50000E+00	4.08930E+04	4.20657E+04
14	6.00000E+00	3.93600E+04	4.06383E+04
15	8.00000E+00	3.72130E+04	3.56097E+04
16	1.60000E+01	2.65330E+04	2.30624E+04
17	2.40000E+01	1.71470E+04	1.69867E+04
18	4.80000E+01	9.94700E+03	9.92034E+03
19	7.20000E+01	5.68000E+03	6.70493E+03
20	9.60000E+01	4.56000E+03	4.60167E+03
21	1.20000E+02	2.90700E+03	3.16282E+03
22	1.68000E+02	2.00000E+03	1.49458E+03
23	3.08000E+02	6.93000E+02	1.67883E+02

TABLE 9 (cont'd): Patient 4

Double Exponential Decay,
Statistical weighting

Variable	Value	Std. Err.
Initial value A	3.49735E+04	1.00250E+03
Rate alpha	2.13183E-01	1.23910E-02
Initial value B	1.06669E+04	7.70802E+02
Rate beta	1.71582E-02	1.00025E-03

	Time (hours)	CPM/ml	Calculated
1	2.50000E-01	4.63700E+04	4.37796E+04
2	5.00000E-01	4.18100E+04	4.20132E+04
3	7.50000E-01	4.04400E+04	4.03363E+04
4	1.00000E+00	3.86900E+04	3.87443E+04
5	1.50000E+00	3.61400E+04	3.57975E+04
6	2.00000E+00	3.14300E+04	3.31404E+04
7	2.50000E+00	2.96100E+04	3.07438E+04
8	3.00000E+00	2.71100E+04	2.85813E+04
9	3.50000E+00	2.62500E+04	2.66293E+04
10	4.00000E+00	2.58000E+04	2.48668E+04
11	4.50000E+00	2.36600E+04	2.32744E+04
12	5.00000E+00	2.24700E+04	2.18352E+04
13	5.50000E+00	2.01300E+04	2.05337E+04
14	6.00000E+00	1.98300E+04	1.93561E+04
15	1.40000E+01	1.12700E+04	1.01574E+04
16	2.20000E+01	7.93000E+03	7.63436E+03
17	3.00000E+01	5.69000E+03	6.43347E+03
18	7.37500E+01	3.16000E+03	3.00937E+03
19	1.01750E+02	1.55200E+03	1.86135E+03
20	1.14000E+02	1.50300E+03	1.50850E+03
21	1.20000E+02	1.26100E+03	1.36093E+03
22	1.44000E+02	9.32000E+02	9.01558E+02
23	1.96000E+02	5.45000E+02	3.69407E+02
24	2.40000E+02	3.44000E+02	1.73633E+02

TABLE 9 (cont'd): Patient 5

Double Exponential Decay,
Statistical weighting

Variable	Value	Std. Err.
Initial value A	2.81153E+04	1.87154E+03
Rate alpha	3.02228E-01	3.97955E-02
Initial value B	1.38336E+04	1.40868E+03
Rate beta	1.98188E-02	1.84091E-03

	Time (hours)	CPM/ml	Calculated
1	2.50000E-01	4.51230E+04	3.98345E+04
2	5.00000E-01	3.66940E+04	3.78693E+04
3	1.00000E+00	3.22760E+04	3.43441E+04
4	1.50000E+00	3.02780E+04	3.12957E+04
5	2.00000E+00	2.80720E+04	2.86574E+04
6	2.50000E+00	2.49920E+04	2.63718E+04
7	3.00000E+00	2.41220E+04	2.43897E+04
8	3.50000E+00	2.25210E+04	2.26687E+04
9	4.00000E+00	2.13340E+04	2.11723E+04
10	4.50000E+00	2.03100E+04	1.98691E+04
11	5.00000E+00	1.93680E+04	1.87323E+04
12	5.50000E+00	1.82950E+04	1.77387E+04
13	6.00000E+00	1.75020E+04	1.68683E+04
14	8.00000E+00	1.51800E+04	1.43108E+04
15	1.60000E+01	1.00750E+04	1.02977E+04
16	2.40000E+01	8.53400E+03	8.61718E+03
17	4.80000E+01	5.45600E+03	5.34306E+03
18	7.20000E+01	2.83800E+03	3.32060E+03
19	9.60000E+01	2.02100E+03	2.06369E+03
20	1.14000E+02	1.01400E+03	1.44449E+03
21	1.68000E+02	9.05000E+02	4.95367E+02
22	2.16000E+02	6.46000E+02	1.91330E+02
23	2.40000E+02	5.55000E+02	1.18908E+02
24	2.64000E+02	5.22000E+02	7.38989E+01

TABLE 9 (cont'd): Patient 6

Double Exponential Decay,
Statistical weighting

Variable	Value	Std. Err.
Initial value A	1.51398E+04	1.05316E+03
Rate alpha	2.69817E-01	3.35793E-02
Initial value B	2.73021E+03	4.79211E+02
Rate beta	4.94487E-03	1.72513E-03

	Time (hours)	CPM/ml	Calculated
1	2.50000E-01	2.18200E+04	1.68791E+04
2	5.00000E-01	1.74320E+04	1.59526E+04
3	7.50000E-01	1.47140E+04	1.50863E+04
4	1.00000E+00	1.31700E+04	1.42763E+04
5	1.50000E+00	1.10180E+04	1.28107E+04
6	2.00000E+00	1.01010E+04	1.15293E+04
7	2.50000E+00	9.37500E+03	1.04088E+04
8	3.00000E+00	8.73300E+03	9.42880E+03
9	3.50000E+00	8.12400E+03	8.57169E+03
10	4.00000E+00	7.73400E+03	7.82193E+03
11	4.50000E+00	7.76200E+03	7.16598E+03
12	5.00000E+00	7.06900E+03	6.59199E+03
13	5.50000E+00	6.65200E+03	6.08963E+03
14	6.00000E+00	6.39300E+03	5.64985E+03
15	8.00000E+00	5.33200E+03	4.37288E+03
16	1.60000E+01	3.78900E+03	2.72448E+03
17	2.40000E+01	2.25000E+03	2.44801E+03
18	4.80000E+01	1.80200E+03	2.15339E+03
19	7.20000E+01	1.59100E+03	1.91238E+03
20	9.60000E+01	1.36100E+03	1.69837E+03
21	1.20000E+02	1.38000E+03	1.50832E+03
22	1.44000E+02	1.42700E+03	1.33953E+03
23	1.96000E+02	1.16300E+03	1.03581E+03
24	2.40000E+02	1.18800E+03	8.33276E+02

TABLE 9 (cont'd): **Patient 6**

Double Exponential Decay, points through 4 days only
Statistical weighting (used for analysis)

Variable	Value	Std. Err.
Initial value A	1.44615E+04	1.22534E+03
Rate alpha	3.89463E-01	6.76874E-02
Initial value B	4.72490E+03	8.27349E+02
Rate beta	1.65940E-02	4.37999E-03

	Time (hours)	CPM/ml	Calculated
1	2.50000E-01	2.18200E+04	1.78252E+04
2	5.00000E-01	1.74320E+04	1.65885E+04
3	7.50000E-01	1.47140E+04	1.54648E+04
4	1.00000E+00	1.31700E+04	1.44437E+04
5	1.50000E+00	1.10180E+04	1.26718E+04
6	2.00000E+00	1.01010E+04	1.12070E+04
7	2.50000E+00	9.37500E+03	9.99499E+03
8	3.00000E+00	8.73300E+03	8.99105E+03
9	3.50000E+00	8.12400E+03	8.15843E+03
10	4.00000E+00	7.73400E+03	7.46687E+03
11	4.50000E+00	7.76200E+03	6.89147E+03
12	5.00000E+00	7.06900E+03	6.41172E+03
13	5.50000E+00	6.65200E+03	6.01075E+03
14	6.00000E+00	6.39300E+03	5.67467E+03
15	8.00000E+00	5.33200E+03	4.77884E+03
16	1.60000E+01	3.78900E+03	3.65158E+03
17	2.40000E+01	2.25000E+03	3.17398E+03
18	4.80000E+01	1.80200E+03	2.13045E+03
19	7.20000E+01	1.59100E+03	1.43057E+03
20	9.60000E+01	1.36100E+03	9.60615E+02

TABLE 9 (cont'd): **Patient 7**

Double Exponential Decay,
Statistical weighting

Variable	Value	Std. Err.
Initial value A	1.33399E+04	8.15015E+02
Rate alpha	2.78876E-01	3.44548E-02
Initial value B	9.55114E+03	7.26960E+02
Rate beta	2.23842E-02	1.58333E-03

	Time (hours)	CPM/ml	Calculated
1	2.50000E-01	2.38700E+04	2.19394E+04
2	5.00000E-01	2.04420E+04	2.10485E+04
3	7.50000E-01	2.02830E+04	2.02144E+04
4	1.00000E+00	2.01970E+04	1.94331E+04
5	1.50000E+00	1.74760E+04	1.80155E+04
6	2.00000E+00	1.59480E+04	1.67700E+04
7	2.50000E+00	1.49320E+04	1.56744E+04
8	3.00000E+00	1.45180E+04	1.47092E+04
9	3.50000E+00	1.37280E+04	1.38578E+04
10	4.00000E+00	1.31750E+04	1.31053E+04
11	4.50000E+00	1.21050E+04	1.24390E+04
12	5.00000E+00	1.21650E+04	1.18479E+04
13	5.50000E+00	1.21100E+04	1.13223E+04
14	6.00000E+00	1.11020E+04	1.08538E+04
15	8.00000E+00	9.97300E+03	9.41815E+03
16	1.60000E+01	6.77200E+03	6.82989E+03
17	2.40000E+01	5.61800E+03	5.59794E+03
18	4.80000E+01	3.02400E+03	3.26163E+03
19	7.20000E+01	1.60800E+03	1.90599E+03
20	9.60000E+01	1.04000E+03	1.11380E+03
21	1.20000E+02	8.85000E+02	6.50872E+02
22	1.68000E+02	5.03000E+02	2.22265E+02
23	2.40000E+02	2.66000E+02	4.43544E+01

TABLE 10

Mean Normalized CPM, Patients 1-7

Double Exponential Decay,
Statistical weighting

Variable	Value	Std. Err.
Initial value A	3.47153E+04	2.38024E+03
Rate alpha	2.12965E-01	2.91310E-02
Initial value B	1.71464E+04	1.86735E+03
Rate beta	1.53114E-02	1.55624E-03

	Time (hours)	CPM/ml	Calculated
1	2.50000E-01	5.69620E+04	4.99962E+04
2	5.00000E-01	5.00330E+04	4.82243E+04
3	7.50000E-01	4.51620E+04	4.65411E+04
4	1.00000E+00	4.44690E+04	4.49422E+04
5	1.50000E+00	4.09150E+04	4.19794E+04
6	2.00000E+00	3.80410E+04	3.93039E+04
7	2.50000E+00	3.55930E+04	3.68868E+04
8	3.00000E+00	3.36970E+04	3.47019E+04
9	3.50000E+00	3.22960E+04	3.27260E+04
10	4.00000E+00	2.65160E+04	3.09380E+04
11	4.50000E+00	3.01260E+04	2.93190E+04
12	5.00000E+00	2.86890E+04	2.78521E+04
13	5.50000E+00	2.72250E+04	2.65219E+04
14	6.00000E+00	2.63000E+04	2.53148E+04
15	8.00000E+00	2.35940E+04	2.14880E+04
16	1.60000E+01	1.68070E+04	1.45708E+04
17	2.40000E+01	1.30110E+04	1.20829E+04
18	3.60000E+01	8.99600E+03	9.89690E+03
19	4.80000E+01	8.01500E+03	8.22349E+03
20	7.20000E+01	4.82500E+03	5.69376E+03
21	9.60000E+01	3.40700E+03	3.94282E+03
22	1.14000E+02	1.95500E+03	2.99305E+03
23	1.20000E+02	2.71200E+03	2.73033E+03
24	1.44000E+02	2.62700E+03	1.89071E+03
25	1.96000E+02	1.98700E+03	8.52792E+02
26	2.40000E+02	1.31100E+03	4.34770E+02
27	2.64000E+02	9.04000E+02	3.01070E+02
28	3.08000E+02	5.56000E+02	1.53491E+02

TABLE 11

Pharmacokinetic parameters calculated from plasma radioactivity

Parameter	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6[§]	Patient 7
A	4.282E+04	3.502E+04	4.329E+04	3.497E+04	2.812E+04	1.446E+04	1.340E+04
alpha	2.455E-01	2.205E-01	1.138E-01	2.132E-01	3.022E-01	3.895E-01	2.789E-01
B	3.885E+04	4.317E+04	2.060E+04	1.067E+04	1.383E+04	4.725E+03	9.551E+03
beta	1.654E-02	1.671E-02	1.562E-02	1.764E-02	1.982E-02	1.659E-02	2.238E-02
	Derived Values						
T(1/2)α	2.82	3.14	6.09	3.25	2.29	1.78	2.49
T(1/2)β	41.91	41.48	44.38	40.40	34.97	41.77	30.97
V(Central) (ml)	4653	3686	3614	2832	2548	3516	4091
V(ss) (ml)	9781	6678	11209	12118	7729	14277	9830
Clearance (ml/hr)	151	105	136	165	135	210	198
	Derived values (corrected for body weight)						
V(Central) (ml/kg)	68.03	49.15	70.32	45.03	38.23	78.13	50.26
V(ss) (ml/kg)	143.00	89.04	218.08	192.65	116.02	317.27	120.77
Clearance (ml/kg*hr)	2.20	1.40	2.64	2.62	2.03	4.66	2.43

The parameters derived from each set of plasma radioactivity data when fit to a two compartment model are shown in the top four rows. The calculated pharmacokinetic parameters describing the distribution and clearance of α_1-PI in these ARDS patients are shown in the succeeding rows as raw data and after correcting for body weight.

[§] Parameters calculated using data points collected for Patient 6 from 0 to 120 hours; later data points suggest artifact due to contraction of the steady-state volume of distribution due, perhaps, to resolution of ascites.

TABLE 12

Pharmacokinetic Parameters for α_1PI in
Patients with and without ARDS

Parameter	ARDS patients (present study) (n = 7)	Emphysema patients (n = 9)
T(1/2)α (hours)	3.12 ± 0.49	6.34 ± .44*
T(1/2)β (hours)	39.14 ± 1.65	97.21 ± 4.04*
Central volume of distribution (ml/kg)	57.02 ± 5.24	53.55 ± 4.54**
Steady state volume of distribution (ml/kg)	170.98 ± 27.53	123.50 ± 9.43**
Clearance (ml/kg*hr)	2.57 ± 0.36	0.816 ± 0.057*

Pharmacokinetic parameters describing α_1PI distribution and clearance in patients with ARDS are compared with the same parameters measured in patients without ARDS. The non-ARDS patients consist of 9 individuals with moderate to severe emphysema who received an infusion of α_1PI (60mg/kg of A1At plus a bolus of radiolabeled α_1PI) under a protocol similar to that used for the present study. These patients include 6 individuals with PiZ α_1PI phenotype and 3 individuals with PiM phenotype.

* $p < 0.002$
** $p > 0.05$

TABLE 13

Results of Analyses of Bronchoaleolar Lavage α_1-PI for Individual Patients Receiving α_1-PI

BAL Analyses

	Albumin (ug/ml)	A1PI Antigenic (ug/ml)	A1PI Activity (ug/ml)	Specific Activity (%)	Complex ng/ml	Infused A1PI ug/ml	Normalized to lavage albumin: A1PI ug/ug alb.	Complex ng/ug alb	Infused A1PI ug/ug alb	Infused A1PI (% total)
Patient 1										
Baseline	18.1	3.53	2.68	75.9%	66.1		0.195	3.654		
6 hour	14.1	3.14	1.45	46.2%	171.3		0.223	12.149		
24 hour	32.1	6.76	4.18	61.8%	207.6	2.49	0.211	6.467	0.078	36.8%
Patient 2										
Baseline	9.51	1.00	1.20	120.0%	150.3		0.105	15.804		
6 hour	19.8	3.20	3.17	99.1%	580.1	3.43	0.162	29.298	0.173	107.1%
24 hour	1.96	0.30	0.00	0.0%	41.6		0.153	21.204		
Patient 3										
Baseline	78.3	33.75	28.80	85.3%	69.8		0.431	0.891		
6 hour	86.1	40.05	36.90	92.1%	331.2	6.23	0.465	3.847	0.072	15.6%
24 hour	40.6	13.20	13.50	102.3%	143.9		0.325	3.544		
Patient 4										
Baseline	14.13	1.88	1.42	75.5%	43.5		0.133	3.079		
6 hour	12.47	1.40	0.63	44.6%	197.4		0.112	15.830		
24 hour	8.89	1.00	0.83	82.7%	95.4		0.112	10.731		
Patient 5										
Baseline	122.1	91.48	89.10	97.4%	144.9		0.749	1.187		
6 hour	91.65	68.34	49.80	72.9%	107.0	16.51	0.746	1.167	0.180	24.2%
24 hour	184.2	103.20	107.60	104.3%	67.9	11.37	0.560	0.369	0.062	11.0%
Patient 6										
Baseline	51.8	23.42	13.20	56.4%	59.9		0.452	1.156		
6 hour	41.85	14.33	8.70	60.7%	11.4	6.62	0.342	0.272	0.158	46.2%
24 hour	17.8	9.84	7.76	78.9%	71.3	6.34	0.553	4.003	0.356	64.4%
Patient 7										
Baseline	68.2	51.90	22.50	43.4%	83.6		0.761	1.226		
6 hour	64.2	14.40	17.95	124.7%	53.0	9.18	0.224	0.825	0.143	63.7%

TABLE 13

TABLE 14

Levels of BAL and Plasma α_1-PI Normalized to Albumin

		Time of sampling		
		Preinfusion	6 hours	24 hours
α_1-PI (μg/μg albumin)	Plasma	0.33 ± 0.06	0.43 ± 0.07	0.35 ± 0.06
	BAL	0.40 ± 0.09	0.33 ± 0.08	0.35 ± 0.08
α_1-PI elastase complex (ng/μg albumin)	Plasma	0.024 ± 0.004	0.021 ± 0.003	0.021 ± 0.004
	BAL	3.86 ± 1.88	9.06 ± 3.78	7.72 ± 2.78

Levels of α_1-PI were measured in lavage samples (or in concurrently obtained plasma samples) obtained during the baseline observation period, and at 6 and 24 hours after the infusion of α_1-PI. The measured levels of α_1-PI in each sample were normalized to the level of albumin present in the same sample and expressed as a ratio. No significant differences in the normalized α_1-PI levels were found between plasma and lavage samples; data are shown as mean ± SEM. In contrast, when levels of α_1-PI in complex with elastase were measured and similarly expresed as a ratio with albumin, lavage fluid levels exceeded plasma levels by two orders of magnitude ($p < 0.001$).

TABLE 15

Results of Analyses of Bronchoaleolar Lavage Cells and Protein for Individual Patients Receiving α_1PI

BAL Analyses

	Vol. (ml) in	Vol. (ml) out	Recovery (%)	Cell recovery (#/ml)	Cell recovery total	PMNs (% total)	Lymph. (% total)	Mono. (% total)	Macro (% total)	Protein (ug/ml)	Protein ug (total)
Patient 1											
Baseline	30	11	36.7%	3.50E+05	3.85E+06	77	2	1	21	576.4	6340.4
6 hour	30	10	33.3%	1.68E+05	1.68E+06	80	0	0	20	83.9	839
24 hour	30	11	36.7%	4.45E+05	4.90E+06	71	1	0	28	167.8	1845.8
Patient 2											
Baseline	30	16	53.3%	2.96E+06	4.74E+07	8	0	0	92	110.1	1761.6
6 hour	30	15	50.0%	8.06E+06	1.21E+08	1	25	0	74	186.7	2800.5
24 hour	30	13	43.3%	2.22E+06	2.89E+07	2	52	0	46	0	0
Patient 3											
Baseline	30	16	53.3%	3.92E+06	6.27E+07	32	1	0	67	440	7040
6 hour	30	15	50.0%	2.87E+06	4.31E+07	64	3	0	34	613.7	9205.5
24 hour	30	10	33.3%	2.50E+06	2.50E+07	64	1	0	35	266.6	2666
Patient 4											
Baseline	30	15	50.0%	2.40E+05	3.60E+06	10	0	0	90	115.2	1728
6 hour	30	15	50.0%	8.00E+05	1.20E+07	55	4	0	41	83.8	1257
24 hour	30	13	43.3%	2.85E+05	3.71E+06	26	0	0	74	67.7	880.1
Patient 5											
Baseline	60	29	48.3%	1.48E+05	4.28E+06	87	4	0	9	564.2	16362
6 hour	60	40	66.7%	7.03E+05	2.81E+07	47	7	0	46	445.8	17832
24 hour	60	25	41.7%	1.30E+05	3.25E+06	42	2	0	56	755.2	18880
Patient 6											
Baseline	60	32	53.3%	1.30E+05	4.16E+06	28	5	0	67	310.1	9923.2
6 hour	60	23	38.3%	6.33E+05	1.46E+07	8	8	0	84	175.1	4027.3
24 hour	60	25	41.7%	2.10E+05	5.25E+06	12	7	0	81	140.4	3510
Patient 7											
Baseline	90	50	55.6%	4.80E+05	2.40E+07	52	1	0	47	362.4	18120
6 hour	90	45	50.0%	5.70E+05	2.57E+07	53	0	0	47	609.2	27414

TABLE 15

TABLE 16

Averaged Results of Analyses of Bronchoaleolar Lavage Cells and Protein for Patients Receiving α_1-PI

	Time of lavage sampling		
	Baseline	4-6 hours after infusion	24 hours after infusion
Cells recovered in lavage (10^7 cells)	2.43 ± 0.93	3.86 ± 1.59	1.18 ± .44
Differential (% total)			
Neutrophils	42.0 ± 10.9	44.0 ± 10.2	36.2 ± 10.4
Lymphocytes	1.9 ± 0.7	6.7 ± 3.0	10.5 ± 7.6
Macrophages	56.1 ± 11.3	49.4 ± 7.8	53.3 ± 7.9
Volume recovered ml (%instilled)	24 ± 5 50.1 ± 2.2)	23 ± 5 (48.3 ± 3.7)	16 ± 3 (40.0 ± 1.5)
Protein content (mg recovered)	8.75 ± 2.28	9.05 ± 3.52	5.55 ± 3.00

Bronchoscopy and bronchoalveolar lavage were performed during the period of baseline observation, and at 4-6 hours and 24 hours after infusion of α_1-PI. Analyses of cell recovery and differential counts, and of protein recovery for each time point are shown here. Data are presented as mean ± SEM.

TABLE 17

Characteristics of Patients Receiving Surfactant

Patient	Etiology of ARDS	Barotrauma after Surfactant Administration	Days Ventilated after Surfactant Administration	Discharge from ICU	Outcome
#1	Sepsis	no	4	yes	survived
#2	Pancreatitis	no	38	yes	died
#3	Aspiration	no	10	yes	survived
#4	Trauma	no	14	no	died
#5	Sepsis	no	33	yes	survived
#6	Pancreatitis	no	34	yes	survived

TABLE 18

Alterations in p_aO_2 after Receiving Surfactant or Placebo

Treatment	Time	#1	#2	#3	#4	#5	#6
Surfactant	p_aO_2 15 min before treatment (torr)	88	87	69	77	89	69
	Highest p_aO_2 after treatment (torr)	103	113	110	73	128	111
	Time of highest p_aO_2 after treatment (min)	5	30	5	30	30	5
	Change in p_aO_2 with treatment (torr)	+15	+26	+41	-4	+39	+42
	Avereage change in p_aO_2 with treatment (torr)	+26.5 p = .04 compared to placebo					
Placebo	p_aO_2 15 min before treatment (torr)	74	169	87	83	174	62
	Highest p_aO_2 after treatment (torr)	57	142	83	109	156	66
	Time of highest p_aO_2 after treatment (min)	30	5	5	5	30	5
	Change in p_aO_2 with treatment (torr)	-17	-27	-4	+26	-18	4
	Avereage change in p_aO_2 with treatment (torr)	-6					

TABLE 19: ARTERIAL BLOOD GAS VALUES IN STUDY PATIENTS

			Treatment #1										Treatment #2							
			-6 hr	-3 hr	-15 min	0	5 min	15 min	30 min	1 hr	2 hr	3 hr	-15 min	0	5 min	15 min	30 min	1 hr	2 hr	3 hr
TREATMENT #1 = PLACEBO; TREATMENT #3 = SURFACTANT																				
Patient																				
#1	pH		7.4	7.41	7.43	7.43	7.43		7.43	7.44	7.39	7.4	7.4	7.41	7.34		7.38	7.39	7.38	7.39
	paO2	(mmHg)	74	72	74	53	53		57	57	90	88	88	72	103		67	83	88	93
	paCO2	(mmHg)	44	38	38	38	39		37	39	42	38	38	38	41		39	37	36	36
	pvO2	(mmHg)	37	35													34	36	38	38
#4	pH		7.44	7.43	7.43	7.43	7.43		7.42	7.43	7.44	7.42	7.41	7.41	7.4		7.4	7.4	7.38	7.4
	paO2	(mmHg)	68	77	83	109	83		103	97	82	77	65	64	62		73	82	63	69
	paCO2	(mmHg)	35	37	38	32	38		39	37	38	39	39	41	41		42	41	43	41
	pvO2	(mmHg)				35	35					36		36			37			
#5	pH		7.38	7.37	7.4	7.39	7.35	7.36	7.37	7.4	7.38	7.37	7.37		7.34	7.36	7.39	7.39	7.4	7.36
	paO2	(mmHg)	95	176	183	174	135	151	156	174	140	89	89		88	107	128	154	206	138
	paCO2	(mmHg)	30	29	31	32	36	35	33	31	36	37	37		38	38	35	34	32	37
	pvO2	(mmHg)	36	38		40					45	42	42		42	44	42	47	41	44
TREATMENT #1 = SURFACTANT; TREATMENT #3 = PLACEBO																				
#2	pH			7.34	7.36	7.34	7.33	7.33	7.31	7.37	7.42		7.43	7.43	7.4	7.43	7.42	7.42	7.43	7.42
	paO2	(mmHg)		63	87	85	73	94	113	129	147		169	128	142	139	131	143	139	164
	paCO2	(mmHg)		32	32	33	35	35	36	31	27		26	24	27	26	27	26	27	27
	pvO2	(mmHg)		30	35	34	34	36	38	38	35		35	34	36	35	35	34	35	35
#3	pH		7.44	7.37	7.38	7.36	7.37	7.36	7.36	7.37	7.38	7.34	7.34		7.33	7.34	7.34	7.36	7.35	7.35
	paO2	(mmHg)	78	68	69	73	110	83	81	73	87	87	87		81	80	81	83	97	85
	paCO2	(mmHg)	33	40	39	40	39	41	41	40	40	44	44		45	44	44	42	41	42
	pvO2	(mmHg)	36	38	39			43			44	48	48			46		43		49
#6	pH		7.48	7.49	7.47		7.48	7.48	7.45	7.46	7.45	7.43	7.43		7.43	7.44	7.43	7.43	7.43	7.42
	paO2	(mmHg)	66	67	69		111	74	68	67	63	62	62		62	66	60	65	65	68
	paCO2	(mmHg)	38	37	38		35	39	40	38	37	40	40		40	38	38	37	38	39
	pvO2	(mmHg)			36		41	40	39	38	37	38	38		38	38	38	37	38	39

TABLE 20: VENTILATION PARAMETER VALUES IN STUDY PATIENTS

			Treatment #1										Treatment #2						
			-6 hr	-3 hr	-15 min	0	5 min	15 min	30 min	1 hr	2 hr	3 hr	-15 min	0	5 min	15 min	30 min	1 hr	2 hr	3 hr
TREATMENT #1 = PLACEBO; TREATMENT #3 = SURFACTANT																				
#1	Tidal volume (mech.)	(L)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	FIO2		0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	PEEP/CPAP	(cm H2O)	7.5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Plateau pressure	(cm H2O)	38	38	38	27	51	41	40	40	35	35	35	34	44	40	38	38	33	33
	Peak pressure	(cm H2O)	41	41	40	39	53	44	40.6	41	42	42	42	41	48.5	45	47	41	42	38
	Effective compliance	(cc gas/mmHg)	33	30	30	45	22	28	29	29	33	33	33	34	26	29	30	30	36	36
#4	Tidal volume (mech.)	(ml)	850	850	850	850	850	850	850	850	850	850	850	850	850	850	850	850	850	850
	FIO2		0.7	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
	PEEP/CPAP	(cm H2O)	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
	Plateau pressure	(cm H2O)			56.7		54.4	57.3	56.2	56.9	61.3	62.3	61.9	61.2	66	59.6	60.9	62	59.6	68.3
	Peak pressure	(cm H2O)	59	59	59.5	59	58.6	57.4	58.4	61.3	59.9	61.3	65.2	63.1	67.2	60.2	61	60.3	60.8	69.6
	Effective compliance	(cc gas/mmHg)	19.3	19.3	20.4	19.3	21.6	20.1	20.6	20.3	18.4	18.0	18.1	18.4	16.7	19.1	18.5	18.1	19.1	15.9
#5	Tidal volume (mech.)	(ml)	1000	1000	1070	1000	1030	1030	1030	1080	1080	1080	1080	1080	1090	1090	1090	1090	1090	1000
	FIO2		0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	PEEP/CPAP	(cm H2O)	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
	Plateau pressure	(cm H2O)	34	30	33		34	32	30	31	33				37	36.8	36.1	39	33	32
	Peak pressure	(cm H2O)	40	35	37	35	36	35	35	36	38.5				41	40.8	40	39	38	41
	Effective compliance	(cc gas/mmHg)	45.5	55.6	51.0	-83.3	46.8	51.5	57.2	56.8	51.4				43.6	44.0	45.2	40.4	51.9	50.0
TREATMENT #1 = SURFACTANT; TREATMENT #2 = PLACEBO																				
#2	Tidal volume (mech.)	(ml)	760	760	750	750	750	800	750	750	750	780	780	780	780	830	750	750	750	750
	FIO2	0.7	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
	PEEP/CPAP	(cm H2O 12.5	18	18	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	Plateau pressure	(cm H2O)			62	62	67	64	62	68	68	62	62	73	71	70	65	67	69	69
	Peak pressure	(cm H2O)	66	66	69	69	73	71	68	72	73	69	69	66	66	66	60	63	63	64
	Effective compliance	(cc gas/mmHg)	16	16	18	18	16	18	18	16	16	19	19	15	15	17	17	16	15	15
#3	Tidal volume (mech.)	(ml)	900	900	900	900	900	900	900	900	900	900		900	900	900	900	900	900	900
	FIO2		0.9	0.7	0.7	* 0.7	0.7	0.7	0.7	0.7	0.7	0.7		0.7	0.7	0.7	0.7	0.7	0.7	0.7
	PEEP/CPAP	(cm H2O)	5	5	5	5	5	5	5	5	5	5		5	5	5	5	5	5	5
	Plateau pressure	(cm H2O)	23.1	23.8	23.7	24.7	26.9	25	26.7	25.2	25.7	25.5		25.5	25.6	25	29.7	23.6	23.6	23.8
	Peak pressure	(cm H2O)	26.1	26.2	25.6	27.6	36.9	29.7	34.1	28.3	32.9	32.3		32.3	30	32.3	31.4	26.8	27.4	29.8
	Effective compliance	(cc gas/mmHg)	50	48	48	46	41	45	41	45	43	44		44	44	45	36	48	48	48
#6	Tidal volume (mech.)	(ml)	1000	1000	1080	1080	1160	1160	1100	1100	1080	1080	1080	1080	1070	1080	1080	1090	1060	1080
	FIO2		0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	PEEP/CPAP	(cm H2O)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Plateau pressure	(cm H2O)	42	41	43	43	59	46.4	46	42	41				43	42	43	42	41	42
	Peak pressure	(cm H2O)	48	47	47	47	79	62	49	46	52				45	44	45	46	45	46
	Effective compliance	(cc gas/mmHg)	31.3	32.3	32.7	32.7	23.7	31.9	30.6	34.4	34.8				32.4	33.8	32.7	34.1	34.2	33.8

TABLE 21: HEMODYNAMIC PARAMETER VALUES IN STUDY PATIENTS

				Treatment #1								Treatment #2							
				-6 hr	-3 hr	-15 min	0	5 min	15 min	30 min	1 hr	2 hr	3 hr	-15 min	0	5 min	15 min	30 min	1 hr	2 hr	3 hr
TREATMENT #1 = PLACEBO; TREATMENT #2 = SURFACTANT																					
#1	pRA mean		(mmHg)	12	10	10		10			8	9	12	12		13			9	10	10
	pPA systolic		(mmHg)	37	44	40		50			48	42	40	40		49			40	40	49
	pPA diastolic		(mmHg)	20	20	15		25			20	19	20	20		20			15	15	23
	pPA mean		(mmHg)	26	28	23		33			29	27	27	27		30			23	23	32
	pPAwedge mean		(mmHg)	18	10	18		22			19	18	18	18		NA			19	20	17
	Cardiac output		(L/min)	8.03	9.03	9.4		7.63			8.99	7.75	7.31	7.31		8.48			8.1	7.98	7.94
	Cardiac index	1.75	(L/min/M^2)	4.59	5.16	5.37		4.36			5.14	4.43	4.18	4.18		4.85			4.63	4.56	4.54
#4	pRA mean		(mmHg)	14	17	18		18			17	16	17	17		17			18	19	17
	pPA systolic		(mmHg)	47	43	42		46			46	42	41	41		35			42	44	48
	pPA diastolic		(mmHg)	31	31	34		33			33	30	31	30		29			35	36	38
	pPA mean		(mmHg)			38		40			39	35	35	36		32			39	40	42
	pPAwedge mean		(mmHg)	18	17	18		17			17	16	16	17		17			18	19	19
	Cardiac output		(L/min)	8.21	5.86	5.81		5.85			4.8	5.25	6.55			6.4			5.9	5.85	4.83
	Cardiac index	1.47	(L/min/M^2)	5.59	3.99	3.95		3.98			3.27	3.57	4.46			4.35			4.01	3.98	3.29
#5	pRA mean		(mmHg)	11	10	8	12	11			13	10	10			10			9	9	10
	pPA systolic		(mmHg)	34	31	31	37	36			36	38				36			36	37	37
	pPA diastolic		(mmHg)	24	22	18	27	27			20	22				22			19	22	17
	pPA mean		(mmHg)	27	25	22	30	30			25	27				25			25	27	20
	pPAwedge mean		(mmHg)	13	11	10	10	10			11	12	14			12			11	11	11
	Cardiac output		(L/min)	8.73	7.2		7.03	8.25			6.63	9.77	8.26			9.38			9.1	8.97	8.47
	Cardiac index	1.95	(L/min/M^2)	4.48	3.69		3.61	4.23			3.40	5.01	4.24			4.81			4.67	4.60	4.34
TREATMENT #1 = SURFACTANT; TREATMENT #2 = PLACEBO																					
#2	pRA mean		(mmHg)	12	13	12		12			10	15		10		14			13	14	13
	pPA systolic		(mmHg)	36	34	34		28			34	34		36		34			36	36	38
	pPA diastolic		(mmHg)	24	23	24		18			22	22		20		20			24	22	23
	pPA mean		(mmHg)	28	30	27.3		21.3			26	26		25		25			28	27	28
	pPAwedge mean		(mmHg)	11	11	13		10			12	12		12		12			14	12	13
	Cardiac output		(L/min)	8.09	7.65	8.18		7.95			6.91	6.12		6.44		7.06			6.83	6.86	6.46
	Cardiac index	BSA=	(L/min/M^2)	4.52	4.27	4.56		4.44			3.86	3.42		3.6		3.9			3.8	3.8	3.6
#3	pRA mean		(mmHg)	2	4	7		8			7	8	7	7		7			6		8
	pPA systolic		(mmHg)	18	26	24		23			24	29	31	31		25			27		28
	pPA diastolic		(mmHg)	12	12	14		15			16	21	15	15		17			18		20
	pPA mean		(mmHg)								18	24	22	22		21			20		21
	pPAwedge mean		(mmHg)	3	5	6		9			9	10	11	11		10			10		8
	Cardiac output		(L/min)	6.06	6.1	7		6.92			6.44		8.01	8.01		8 37			6.63		7.6
	Cardiac index	1.72	(L/min/M^2)	3.52	3.55	4.07		4.02			3.74		4.66	4.66		4.87			3.85		4.42
#6	pRA mean		(mmHg)	10		14		12			12	12	12	12		10			11	11	10
	pPA systolic		(mmHg)	35		35		37			39	34	36	36		37			36	34	39
	pPA diastolic		(mmHg)	23		25		22			23	17	21	21		21			20	22	23
	pPA mean		(mmHg)	27		28		27			28	23				26			25	26	28
	pPAwedge mean		(mmHg)	13		14		13			12	14	10	10		13			12	12	12
	Cardiac output		(L/min)	8.79		10.04		9.92			9.68	7.85	9.4	9.4		9.9			10.2	8.8	8.54
	Cardiac index	2.18	(L/min/M^2)	4.03		4.61		4.55			4.44	3.60	4.31	4.31		4.54			4.68	4.04	3.92

TABLE 22

Absorbance Values for ELISA Determination of
Surfactant-Anti-surfactant Immune Complexes

Patient #	Time	Serum Dilution				
		1:1	1:2	1:4	1:8	1:16
#1	Baseline	0	0	0	0	0
	1 week	0	0	0	0.031	0
#2	Baseline	0	0	0	0	0
	1 month	0	0	0	0	0
#3	Baseline	0	0	0	0	0
	2 weeks	0	0	0	0	0
	3.5 months	0	0	0	0	0
#5	Baseline	0	0	0	0	0
	1 month	0.009	0	0	0	0.009
#6	Baseline	0	0	0	0	0
	1 month	0	0	0.004	0.006	0

TABLE 23

Absorbance Values for ELISA Determination of
Free Anti-surfactant Antibodies

Patient #	Time	Serum Dilution				
		1:1	1:2	1:4	1:8	1:16
#1	Baseline	0	0.007	0.008	0.006	0.012
	1 week	0.026	0.027	0.003	0.012	0.034
#2	Baseline	0.008	0.001	0.004	0	0.002
	1 month	0	0.012	0.013	0.003	0.005
#3	Baseline	0	0	0.003	0.008	0.112
	2 weeks	0	0	0	0.006	0.081
	3.5 months	0	0	0	0.005	0.049
#5	Baseline	0.004	0.004	0.007	0	0
	1 month	0	0	0	0	0
#6	Baseline	0	0	0	0.005	0
	1 month	0.002	0	0.006	0	0

TABLE 24

Cell Count and Differential of Bronchoalveolar Lavage Fluids Obtained from Patients Receiving Surfactant

#	BAL	Treatment	Site	Vol(ml)	Cells/ml	Segs	Lymph	Macro
#1	Baseline		LLL	17.5	215909	26	4	78
	After Rx 1	Placebo	RLL	15.5	136667	43	7	50
	After Rx 2	Surfactant	Lingula	16	220312	66	2	32
	After 1 day		RUL	15	166667	11	0	89
#2	Baseline		RML	13.3	210000	89	0	11
	After Rx 1	Surfactant	Lingula	9	320000	59	2	39
	After Rx 2	Placebo	LUL	9.3	260000	77	3	20
	After 1 day				125000	93	0	7
#3	Baseline		RML	19	1570000	90	0	10
	After Rx 1	Surfactant	Lingula	10	685000	81	1	18
	After Rx 2	Placebo	RML	10	2120000	95	0	5
	After 1 day							
#4	Baseline		RML			80	0	20
	After Rx 1	Placebo	Lingula	22		71	2	27
	After Rx 2	Surfactant	RML			91	0	9
	After 36 hr		Lingula			93	0	7
#5	Baseline		RML	15	230000	34		66
	After Rx 1	Placebo	RUL	20	258000	34		66
	After Rx 2	Surfactant	LLL	25	160000	43	1	56
	After 1 day		RML	15	310000	25		75
#6	Baseline		RML	23	461700	88		12
	After Rx 1	Surfactant	Lingula	15	576600	89	2	9
	After Rx 2	Placebo		19	136700	97		3
	After 1 day							

TABLE 25

Cell Count and Differential of Bronchoalveolar Lavage Fluids Obtained from Patients Receiving Surfactant

#	BAL	Vol(ml)	Protein (mg/ml)	alPI (mg/ml)	alPI Activity (mg/ml)	alPI Specific Activity	Albumin (mg/ml)	alPI-HNE Complex (ng/ml)	HNE Activity
#1	Baseline	17.5	2650	544	542	100	1048	300.5	0
	After Rx 1	15.5	297	45.1	30.4	67	85.4	148.8	0
	After Rx 2	16	356	42.8	33.4	78	42.7	215.4	0
	After 1 day	15	207	31.2	25.5	82	86.8	141.5	0
#2	Baseline	13.3	1464	118.6	113.2	95	1070	270.9	0
	After Rx 1	9	1402	112.3	117.6	104	989	157.1	0
	After Rx 2	9.3	1747	150.1	130.9	87	1278	160.6	0
	After 1 day		617.5	52.75	38.87	67	234.7	248.8	0
#3	Baseline	19		53.7	qns		268	qns	0
	After Rx 1	10		102.9	qns		553	qns	0
	After Rx 2	10		99.2	qns		301	qns	0
	After 1 day								
#4	Baseline		203.2	23.9	12.69	53	50.96	qns	qns
	After Rx 1	22	563.1	59.12	43.7	74	132.6	qns	qns
	After Rx 2		377.4	76.15	70.8	93	189.7	qns	qns
	After 36 hr		65.36	4.54	2.41	53	13.17	qns	qns
#5	Baseline	15	1540	125.2	113.3	90	555.5	165.6	0
	After Rx 1	20	1260	115.6	113.3	98	444.5	159.9	0
	After Rx 2	25	1780	191.4	162.4	85	786	302.3	0
	After 1 day	15	1880	165.6	154.6	93	593.25		0
#6	Baseline	23	1910	226.4	147.6		795.6	1208	0
	After Rx 1	15	910	101.7	93.9	92	344.4	870.7	0
	After Rx 2	19	550	62.7	62.2	99	212.7	328.9	0
	After 1 day								

TABLE 26

Phospholipid Analysis of Bronchoalveolar Lavage Fluids Obtained from Patients Receiving Exogenous Surfactant

Patient	BAL	Treatment	Site	Volume Recovered	PL conc. (ug/ml)	Total PL (ug)
#1	Baseline		LLL	17.5	41.1	719
	After Rx 1	Placebo	RLL	15.5	20.7	321
	After Rx 2	Surfactant	Lingula	16	188	3008
	After 1 day		RUL	15	13.5	203
#2	Baseline		RML	9	25.5	230
	After Rx 1	Surfactant	Lingula	9	253	2277
	After Rx 2	Placebo	LUL	9	127.4	1147
	After 1 day				171.7	
#3	Baseline		RUL	19	58	1102
	After Rx 1	Surfactant	Lingula	10	317	3170
	After Rx 2	Placebo	RML	10	56	560
	After 1 day					
#4	Baseline		RML	12	15.6	187
	After Rx 1	Placebo	Lingula	22	29.8	656
	After Rx 2	Surfactant	RML		14.6	0
	After 36 hr		Lingula		8.5	
#5	Baseline		RML	15	16.5	248
	After Rx 1	Placebo	RUL	20	21.8	436
	After Rx 2	Surfactant	LLL	25	50.1	1253
	After 1 day		RML	15	19.5	293
#6	Baseline		RML	10	109.6	1096
	After Rx 1	Surfactant	Lingula	6	207.8	1247
	After Rx 2	Placebo		13	21.5	280
	After 1 day					

TABLE 27

Phospholipid Profile of Bronchoalveolar
Lavage Fluid Phospholipids in Lavage Fluids
Obtained from Patients Receiving Exogenous Surfactant

Patient	BAL	Treatment	% of Total PL						
			PC	PG	PE	PI	PS	SM	LPC
#1	Baseline		73.38	7.47				19.15	
	After Rx 1	Placebo							
	After Rx 2	Surfactant							
	After 1 day		14.57	33.51			31.43	20.49	
#2	Baseline								
	After Rx 1	Surfactant							
	After Rx 2	Placebo							
	After 1 day								
#3	Baseline								
	After Rx 1	Surfactant							
	After Rx 2	Placebo							
	After 1 day		63.64	2.20				0.00	
#4	Baseline								
	After Rx 1	Placebo							
	After Rx 2	Surfactant							
	After 36 hr								
#5	Baseline								
	After Rx 1	Placebo							
	After Rx 2	Surfactant	62.80	3.00	9.40	9.10	3.10	9.10	3.50
	After 1 day								
#6	Baseline		61.20	3.60	9.60	5.80	4.30	12.90	2.60
	After Rx 1	Surfactant	32.50	7.40	18.30	16.90	5.40	14.80	4.70
	After Rx 2	Placebo							
	After 1 day								

TABLE 28

Surfactant Activity in Non-extracted or Extracted Bronchoalveolar Lavage Samples Recovered from Patients Receiving Exogenous Surfactant

			Minimum Surface Tension at 5 Minutes (mN/M)		
Patient	BAL	Treatment	Non-extracted	Extracted	Difference
#1	Baseline		34.70	17.60	17.10
	After Rx 1	Placebo			
	After Rx 2	Surfactant	22.70	23.70	-1.00
	After 1 day				
#2	Baseline		23.50	13.70	9.80
	After Rx 1	Surfactant	14.90	13.10	1.80
	After Rx 2	Placebo	15.70	16.10	-0.40
	After 1 day		13.30	15.60	-2.30
#3	Baseline		30.40	17.80	12.60
	After Rx 1	Surfactant	19.60	21.50	-1.90
	After Rx 2	Placebo			
	After 1 day				
#4	Baseline		29.00	24.20	4.80
	After Rx 1	Placebo	31.40	21.10	10.30
	After Rx 2	Surfactant	28.20	21.90	6.30
	After 36 hr				
#5	Baseline		37.80	25.40	12.40
	After Rx 1	Placebo	34.70	27.20	7.50
	After Rx 2	Surfactant	37.40	23.90	13.50
	After 1 day		37.40	28.80	8.60
#6	Baseline		35.40	22.00	13.40
	After Rx 1	Surfactant	31.10	21.70	9.40
	After Rx 2	Placebo	35.50	25.60	9.90
	After 1 day				

APPENDIX II: FIGURES

FIGURE 1

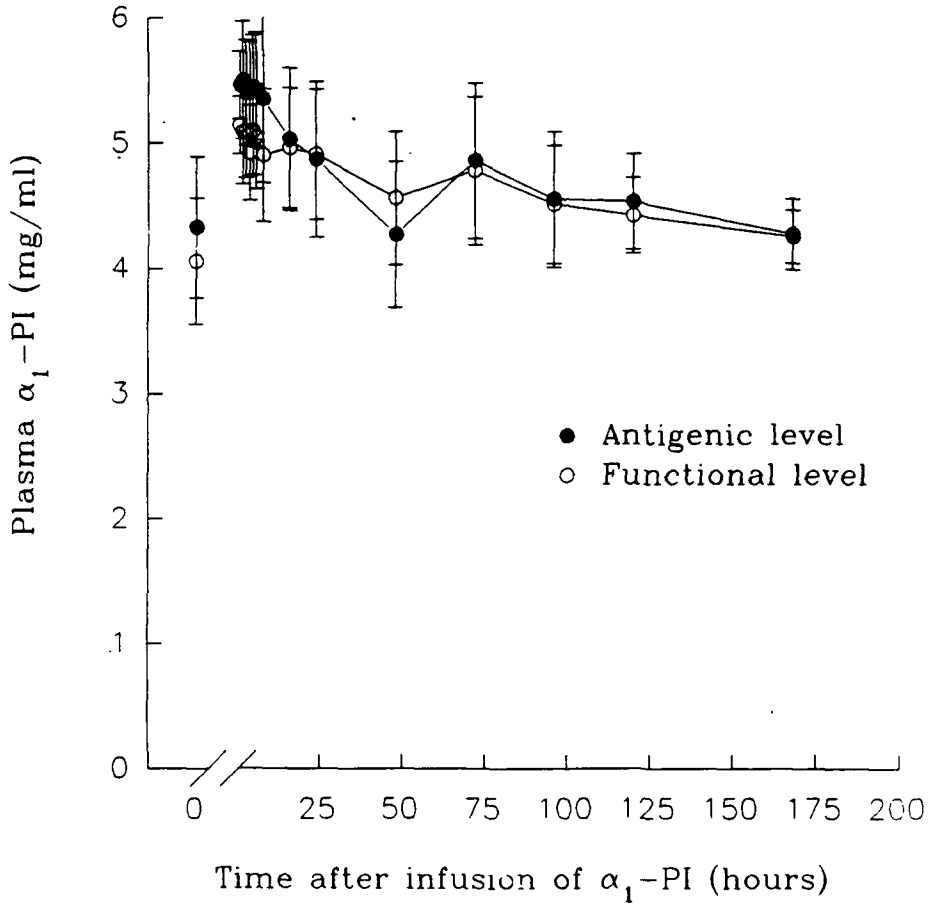

Plasma α_1-PI (mg/ml)
6
5
4
3
2
1
0
Antigenic level
Functional level
0
25
50
75
100
125
150
175
200
Time after infusion of α_1-PI (hours)

FIGURE 2

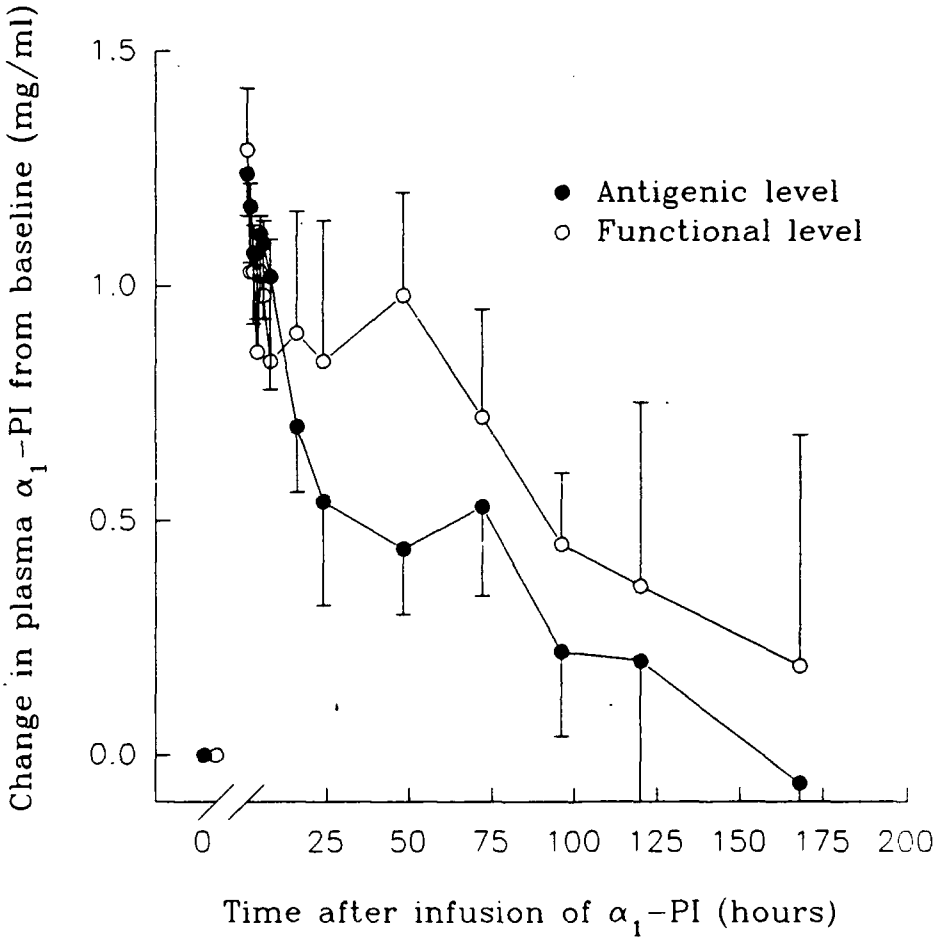

Change in plasma α_1-PI from baseline (mg/ml)
1.5
1.0
0.5
0.0
Antigenic level
Functional level
0
25
50
75
100
125
150
175
200
Time after infusion of α_1-PI (hours)

FIGURE 3

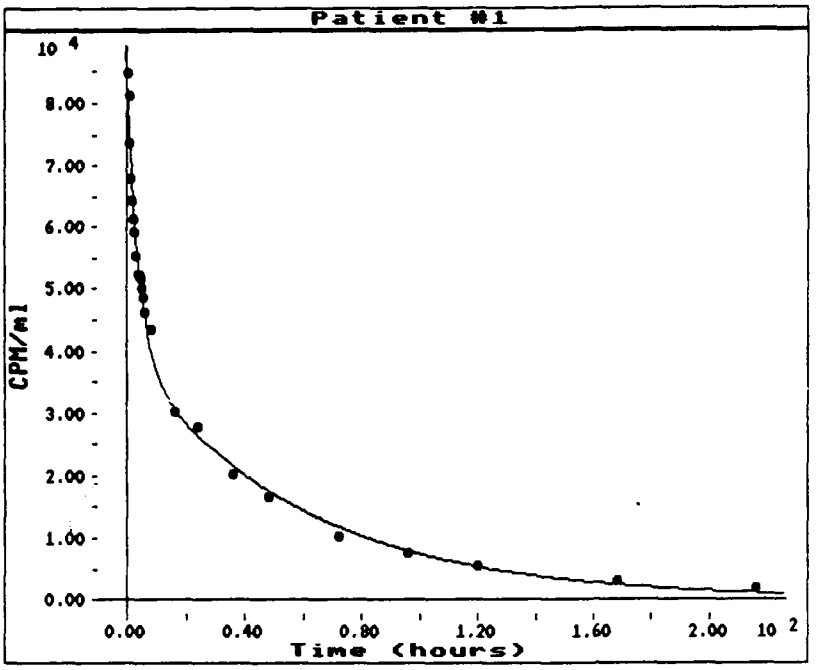

Patient #1
10 4
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
CPM/ml
0.00
0.40
0.80
1.20
1.60
2.00
10 2
Time (hours)

FIGURE 4

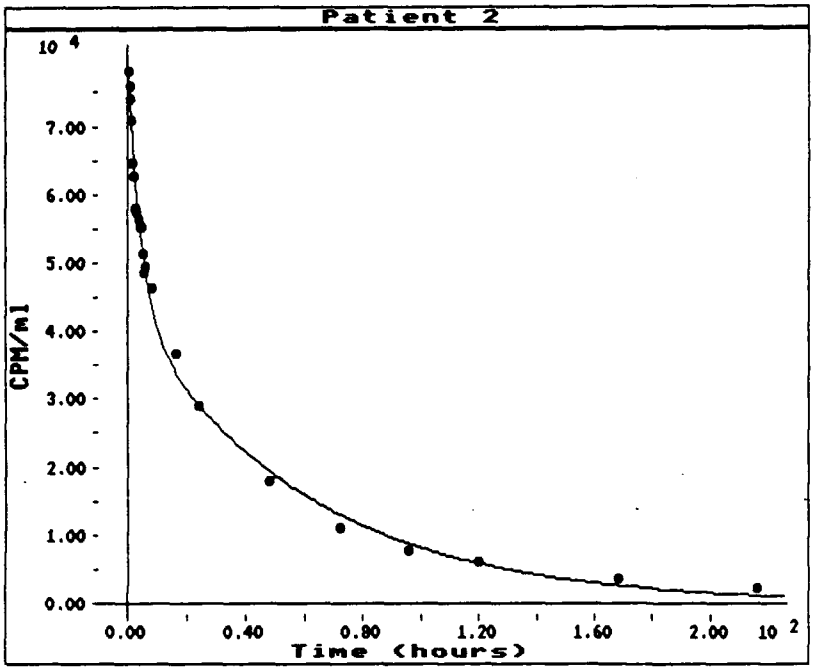

Patient 2
10 4
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
CPM/ml
0.00
0.40
0.80
1.20
1.60
2.00
10 2
Time (hours)

FIGURE 5

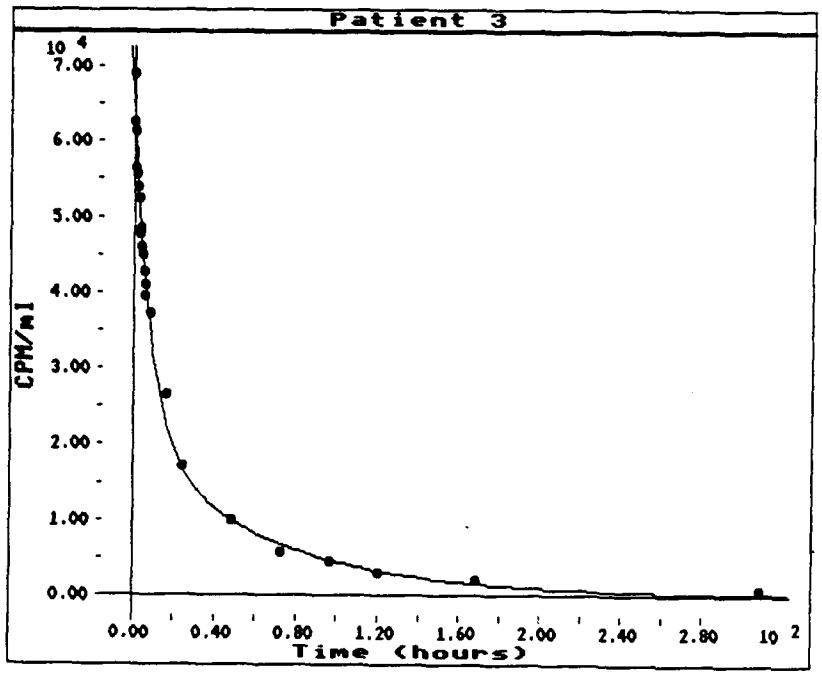

Patient 3
10 4
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
CPM/ml
0.00
0.40
0.80
1.20
1.60
2.00
2.40
2.80
10 2
Time (hours)

FIGURE 6

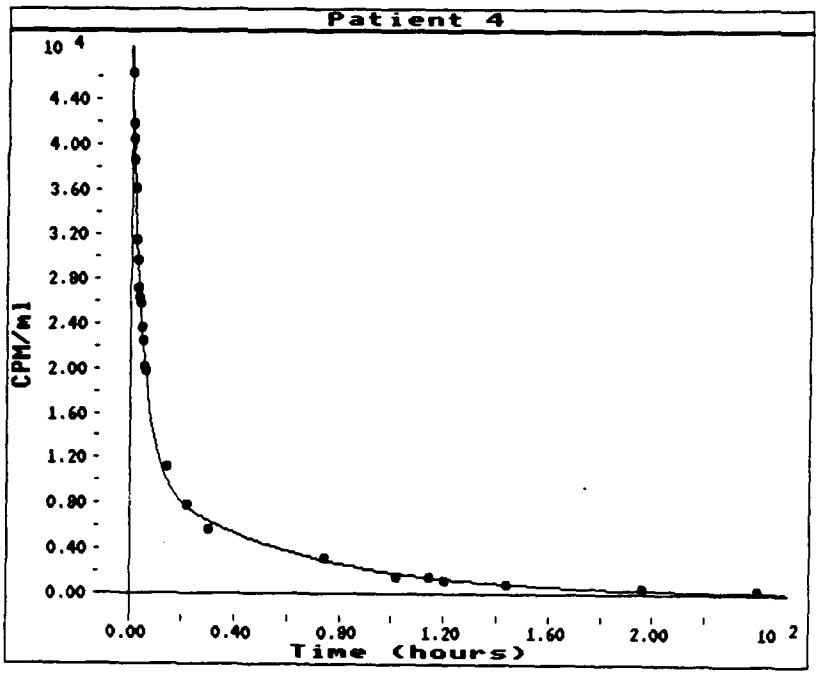

Patient 4
10 4
4.40
4.00
3.60
3.20
2.80
2.40
2.00
1.60
1.20
0.80
0.40
0.00
CPM/ml
0.00
0.40
0.80
1.20
1.60
2.00
10 2
Time (hours)

FIGURE 7

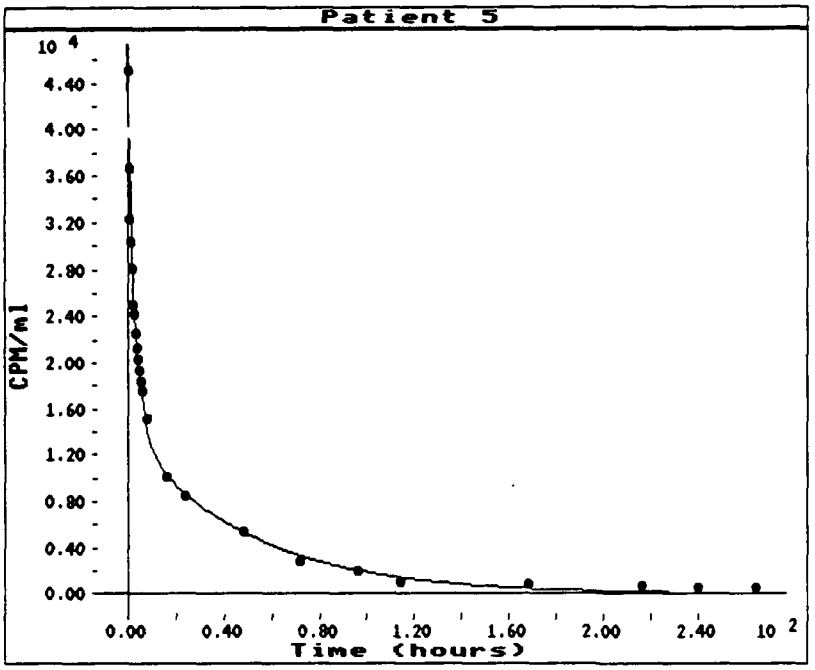

Patient 5
10 4
4.40
4.00
3.60
3.20
2.80
2.40
2.00
1.60
1.20
0.80
0.40
0.00
CPM/ml
0.00
0.40
0.80
1.20
1.60
2.00
2.40
10 2
Time (hours)

FIGURE 8

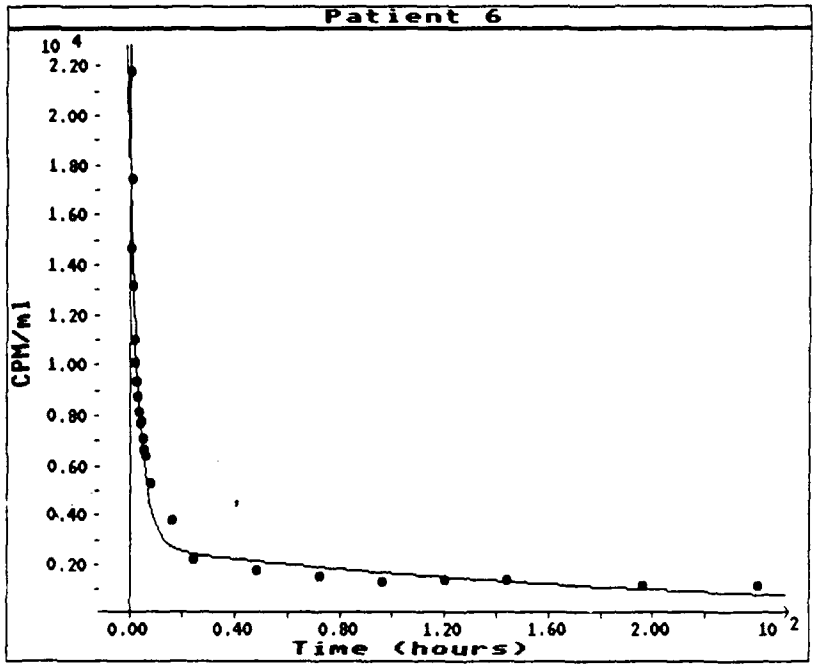

Patient 6
10 4
2.20
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
CPM/ml
0.00
0.40
0.80
1.20
1.60
2.00
10 2
Time (hours)

FIGURE 9

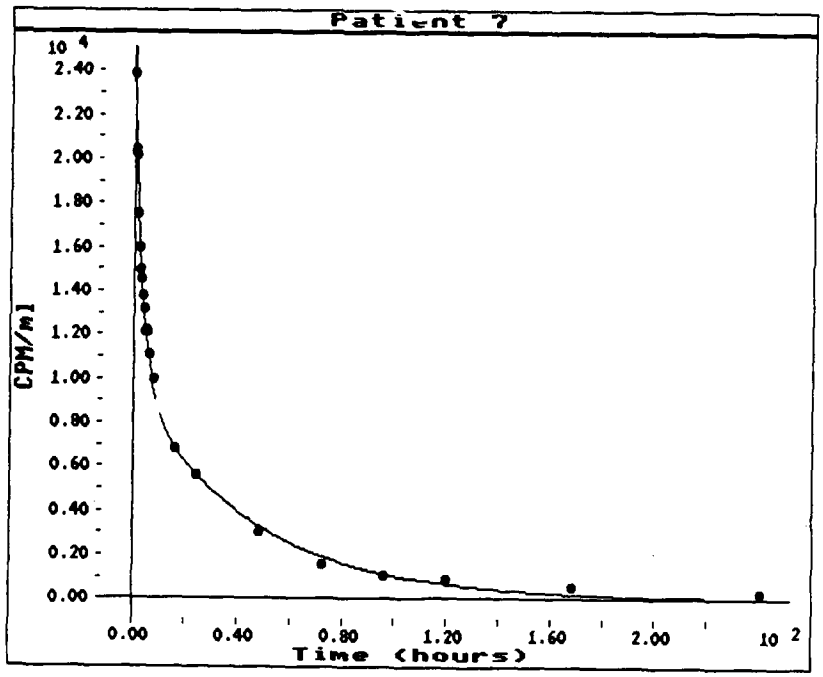

Patient 7
10 4
2.40
2.20
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
CPM/ml
0.00
0.40
0.80
1.20
1.60
2.00
10 2
Time (hours)

FIGURE 10

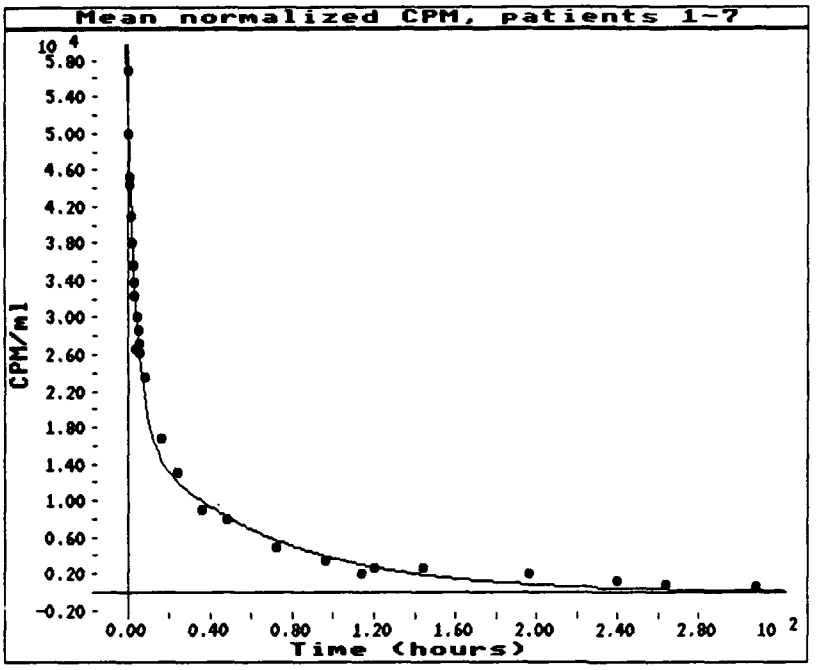

Mean normalized CPM, patients 1-7
10 4
5.80
5.40
5.00
4.60
4.20
3.80
3.40
3.00
2.60
2.20
1.80
1.40
1.00
0.60
0.20
-0.20
CPM/ml
0.00
0.40
0.80
1.20
1.60
2.00
2.40
2.80
10 2
Time (hours)

FIGURE 11

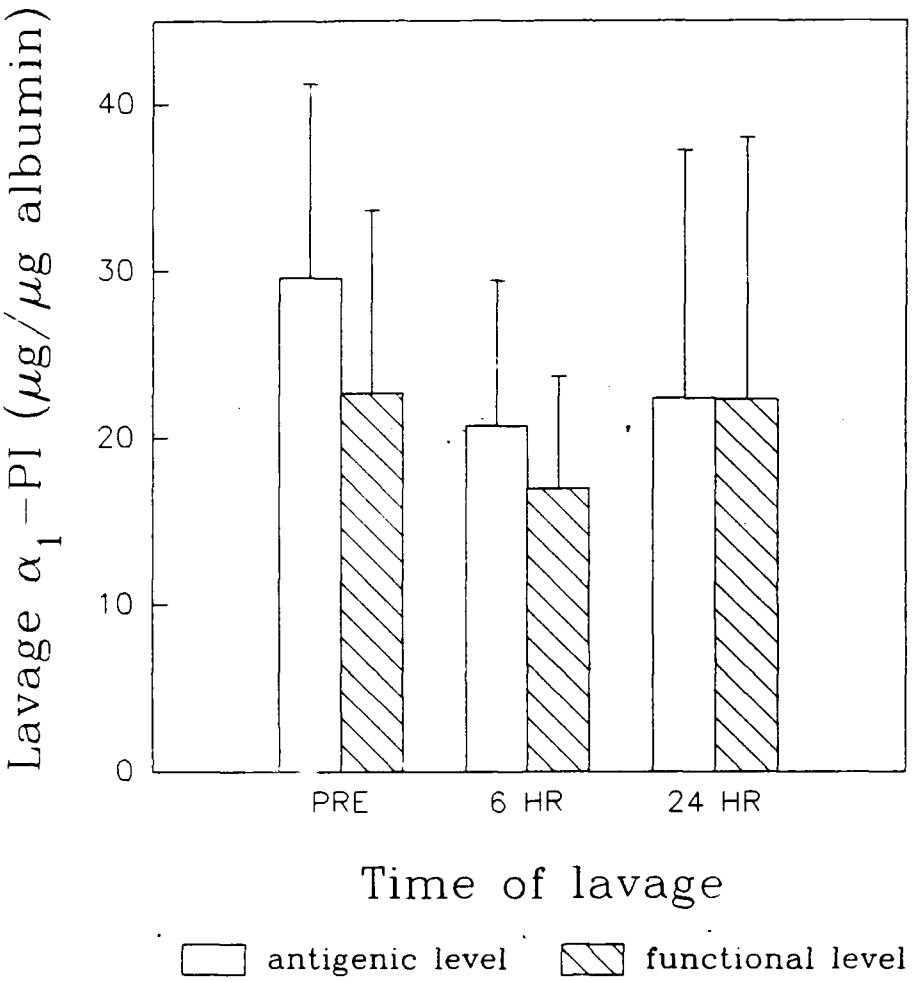

Lavage α_1-PI ($\mu g/\mu g$ albumin)
40
30
20
10
0
PRE
6 HR
24 HR
Time of lavage
antigenic level
functional level

FIGURE 12A

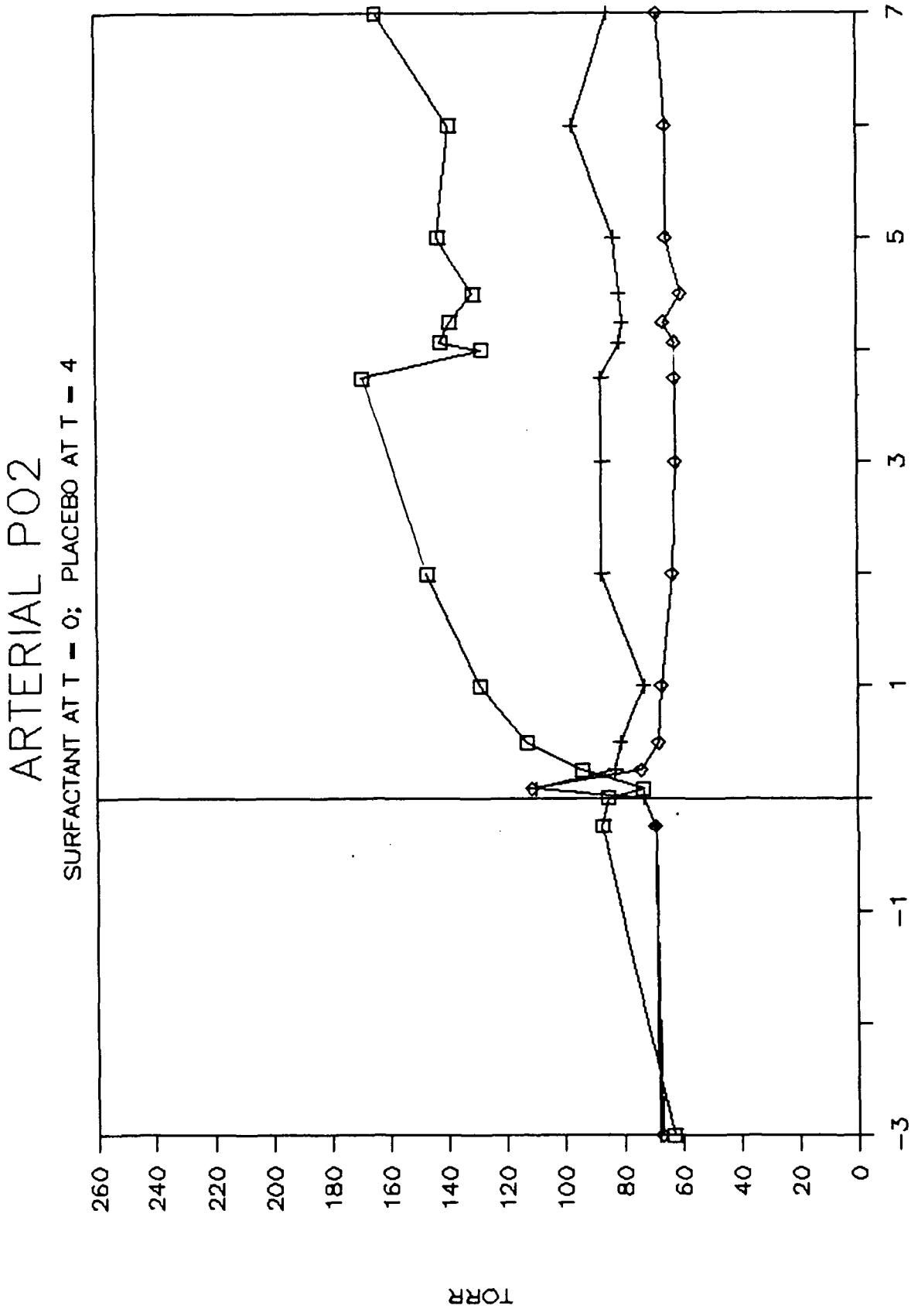

ARTERIAL PO2
SURFACTANT AT T = 0; PLACEBO AT T = 4
TORR
260
240
220
200
180
160
140
120
100
80
60
40
20
0
-3
-1
1
3
5
7
HOURS
□ #2
+ #3
◇ #6

FIGURE 12B

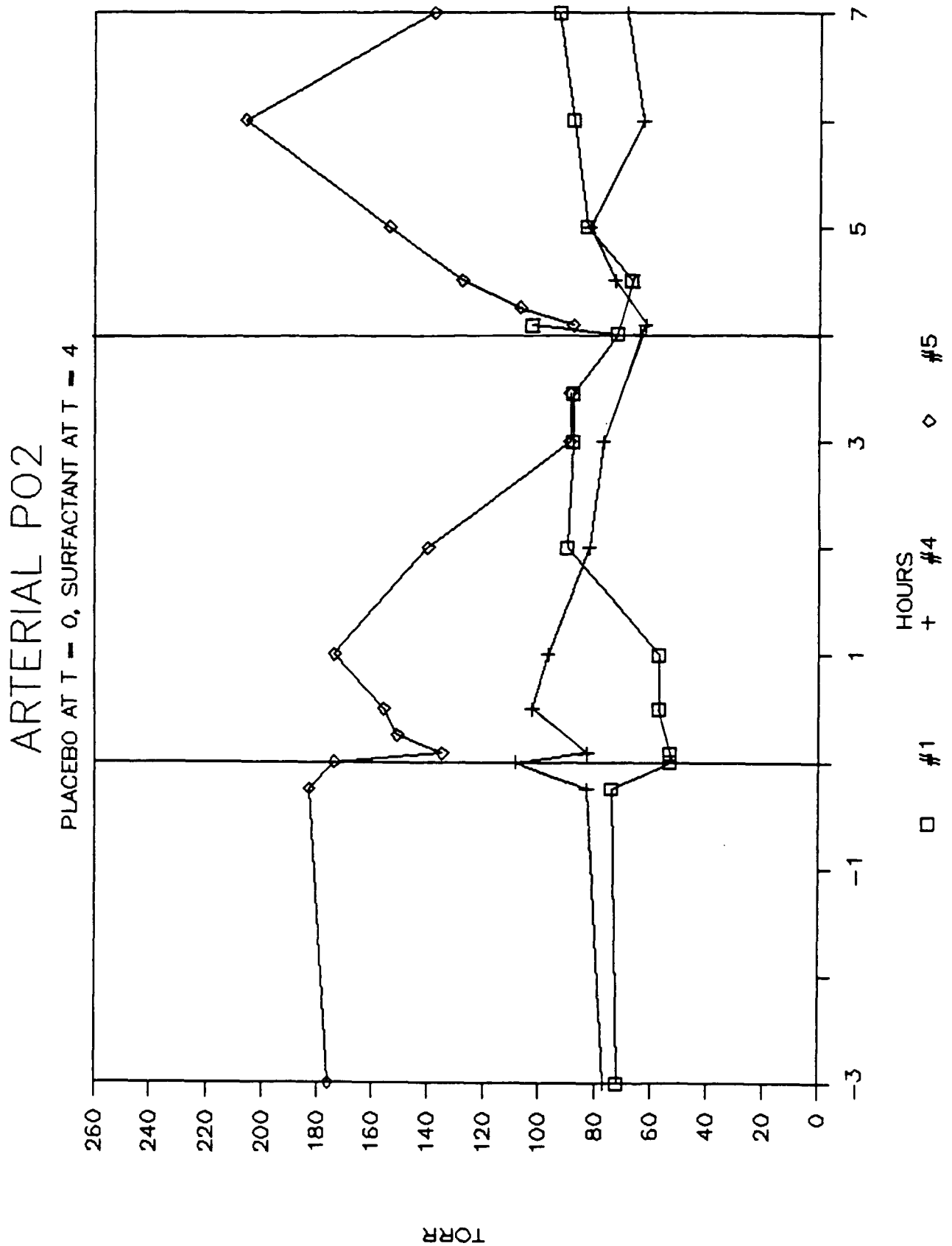

ARTERIAL PO2
PLACEBO AT T = 0, SURFACTANT AT T = 4
TORR
260
240
220
200
180
160
140
120
100
80
60
40
20
0
-3
-1
1
3
5
7
HOURS
□ #1
+ #4
◇ #5

FIGURE 13

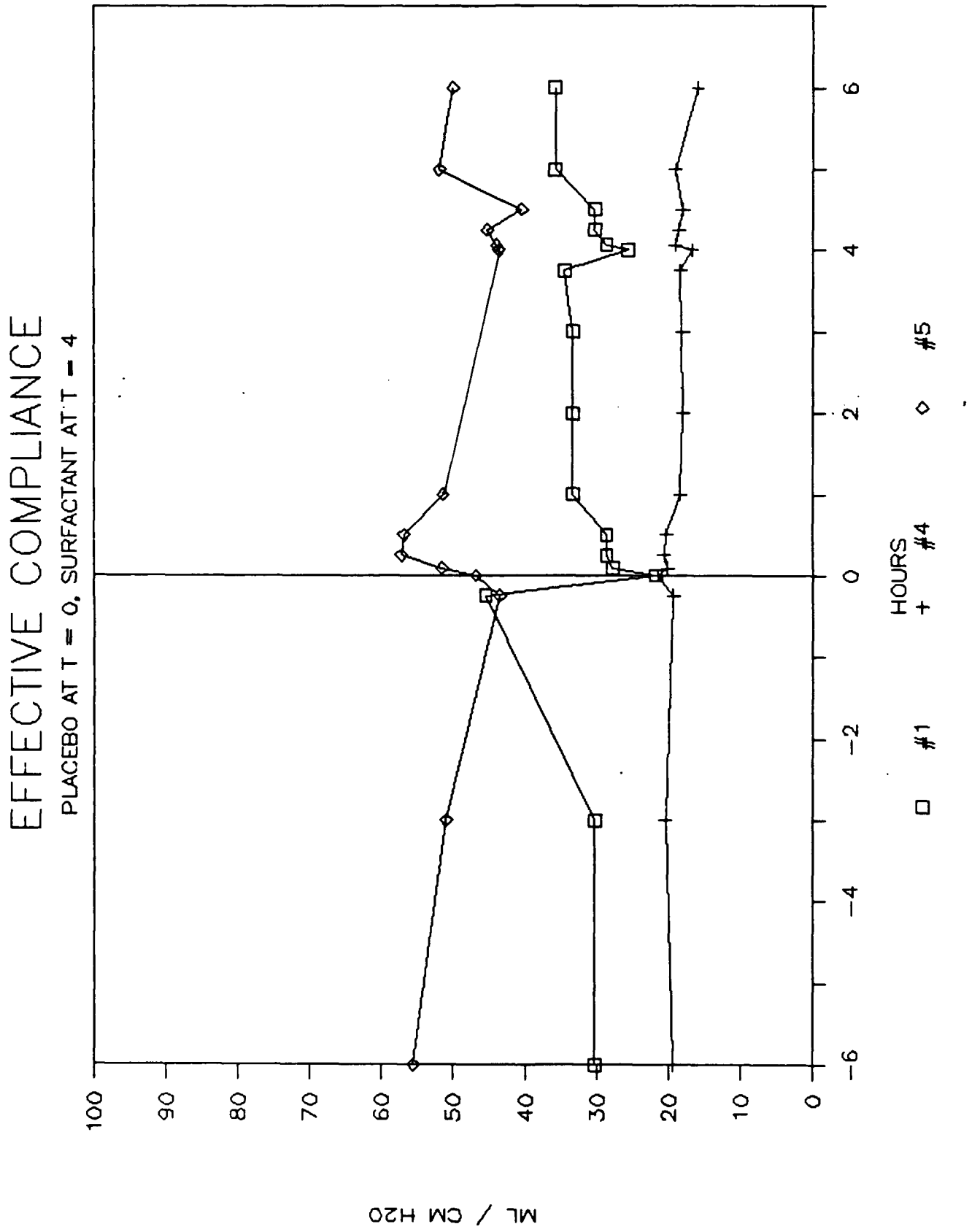

EFFECTIVE COMPLIANCE
PLACEBO AT T = 0, SURFACTANT AT T = 4
ML / CM H2O
HOURS
#1
#4
#5
100
90
80
70
60
50
40
30
20
10
0
-6
-4
-2
0
2
4
6

FIGURE 14

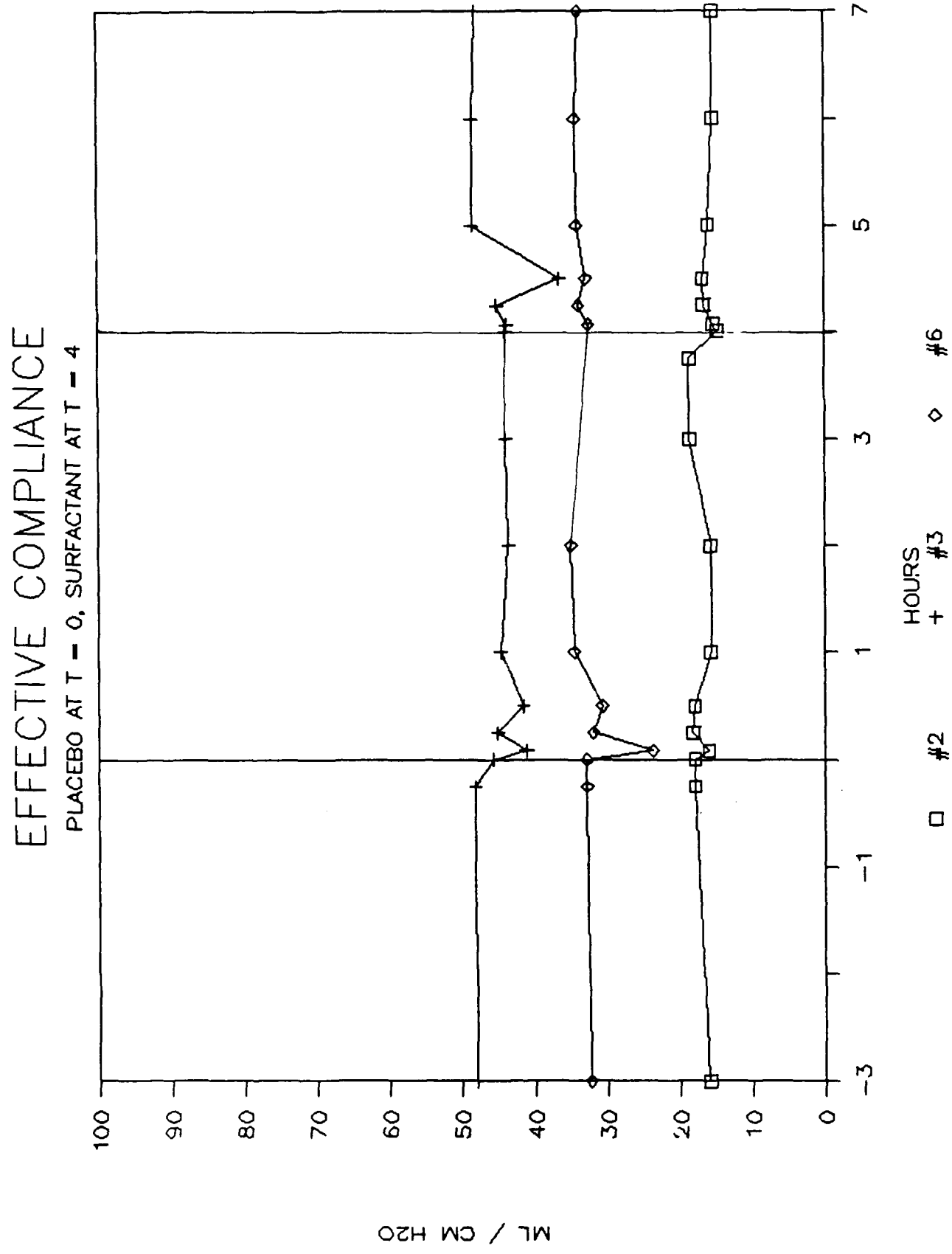

EFFECTIVE COMPLIANCE
PLACEBO AT T = 0, SURFACTANT AT T = 4
ML / CM H2O
100
90
80
70
60
50
40
30
20
10
0
-3
-1
1
3
5
7
HOURS
□ #2
+ #3
◇ #6

FIGURE 15

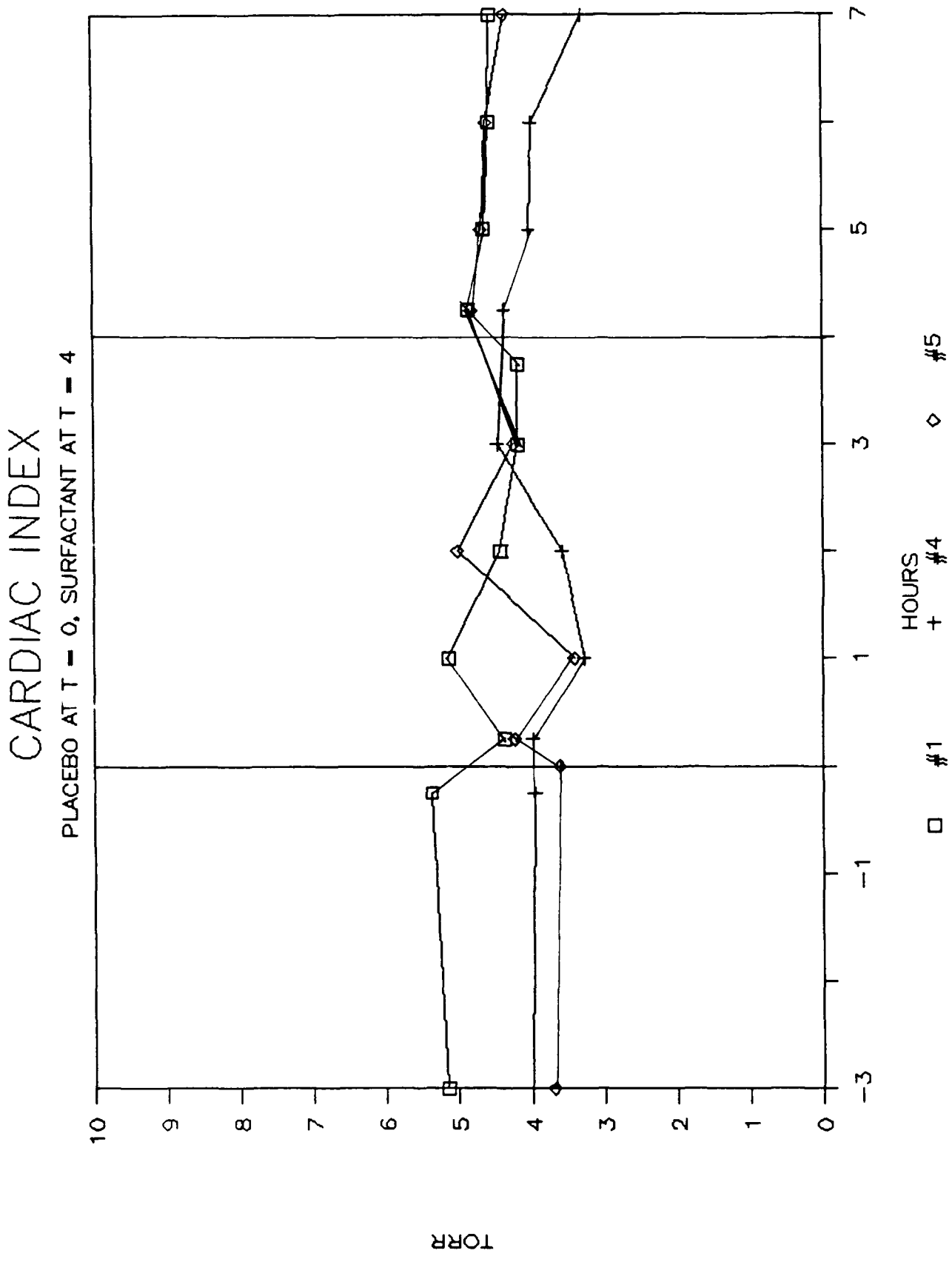

CARDIAC INDEX
PLACEBO AT T = 0, SURFACTANT AT T = 4
HOURS
□ #1
+ #4
◇ #5
-3
-1
1
3
5
7
0
1
2
3
4
5
6
7
8
9
10
TORR

FIGURE 16

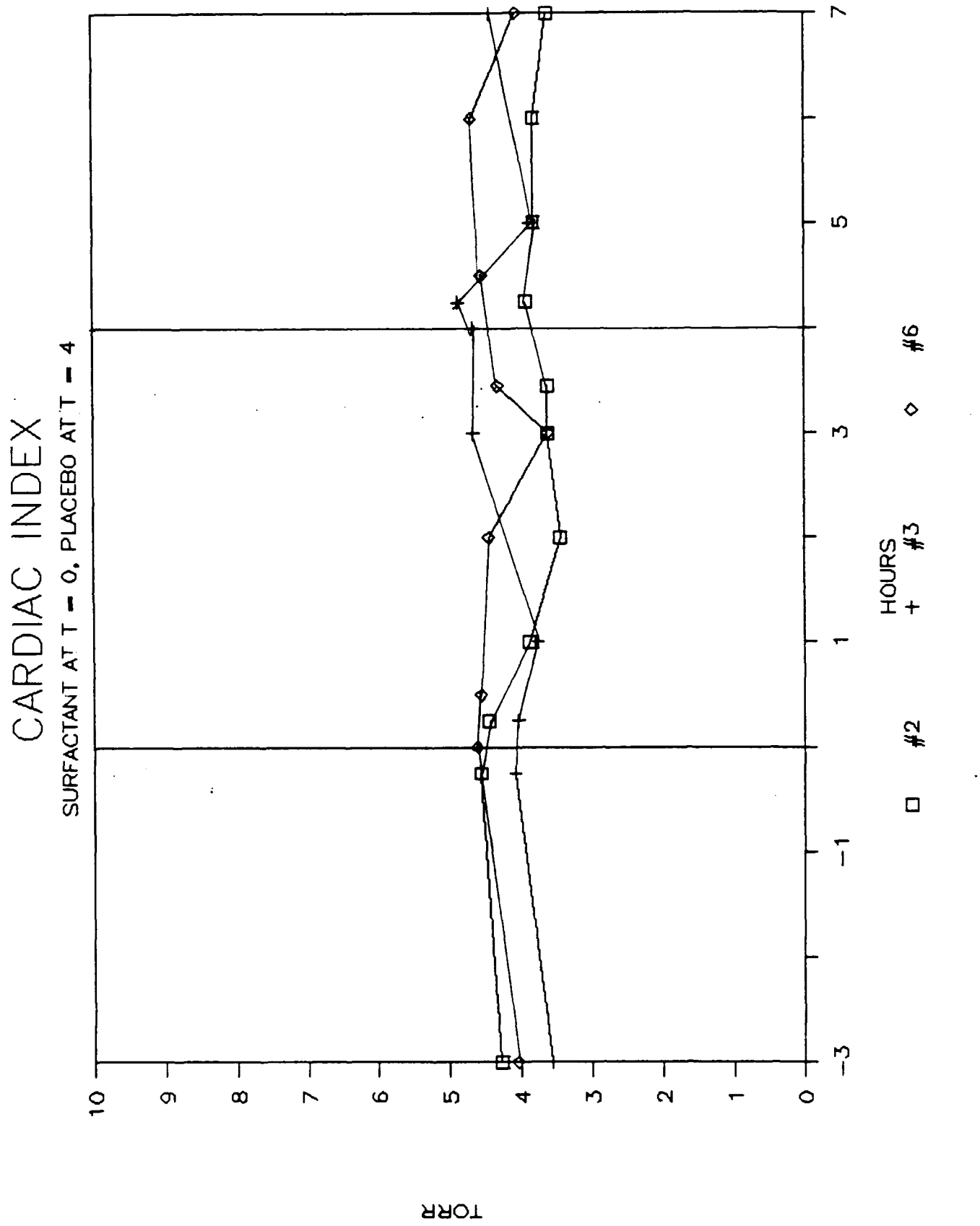

CARDIAC INDEX
SURFACTANT AT T = 0, PLACEBO AT T = 4
TORR
10
9
8
7
6
5
4
3
2
1
0
-3
-1
1
3
5
7
HOURS
□ #2
+ #3
◇ #6

FIGURE 17

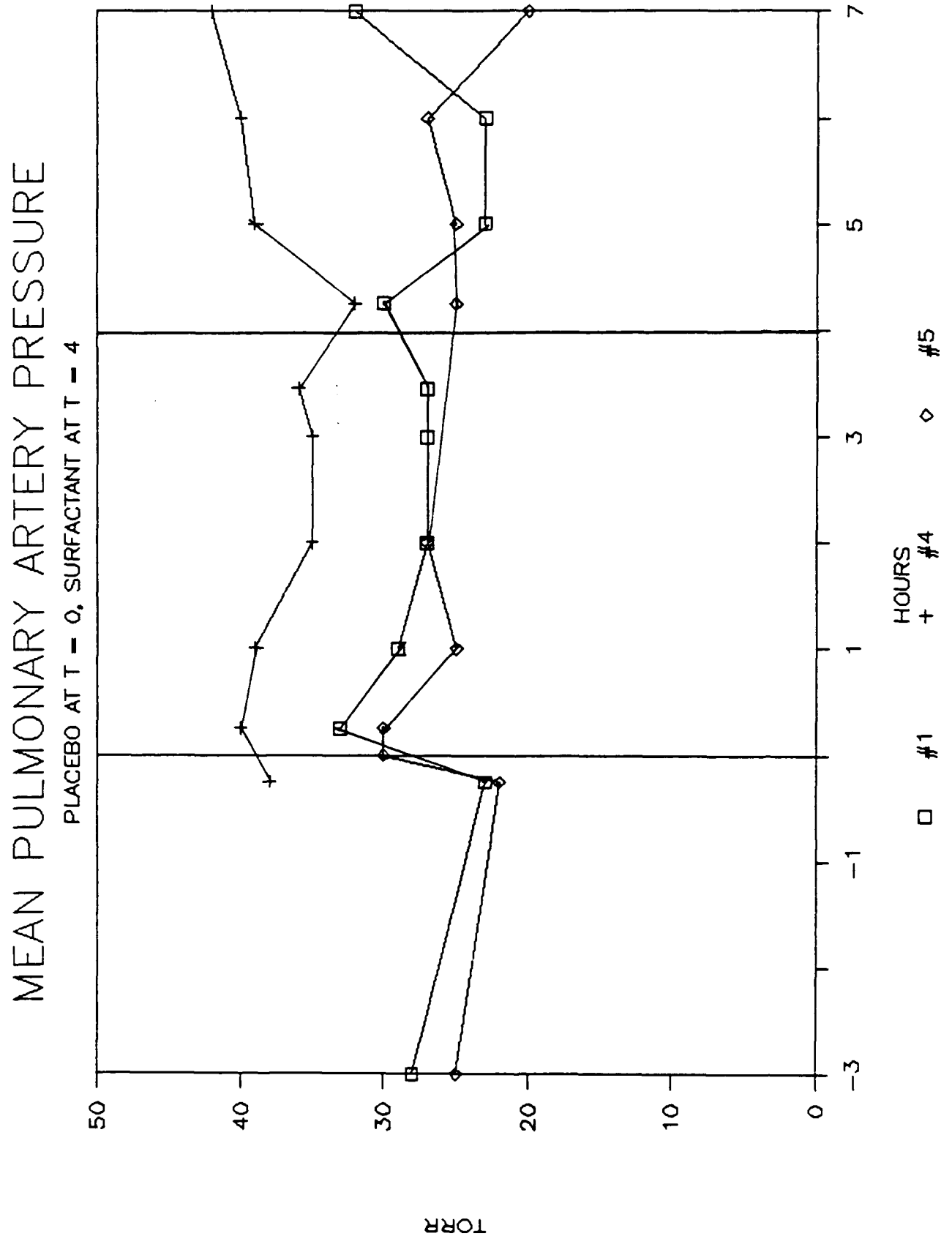

MEAN PULMONARY ARTERY PRESSURE
PLACEBO AT T = 0, SURFACTANT AT T = 4
TORR
50
40
30
20
10
0
-3
-1
1
3
5
7
HOURS
□ #1
+ #4
◇ #5

FIGURE 18

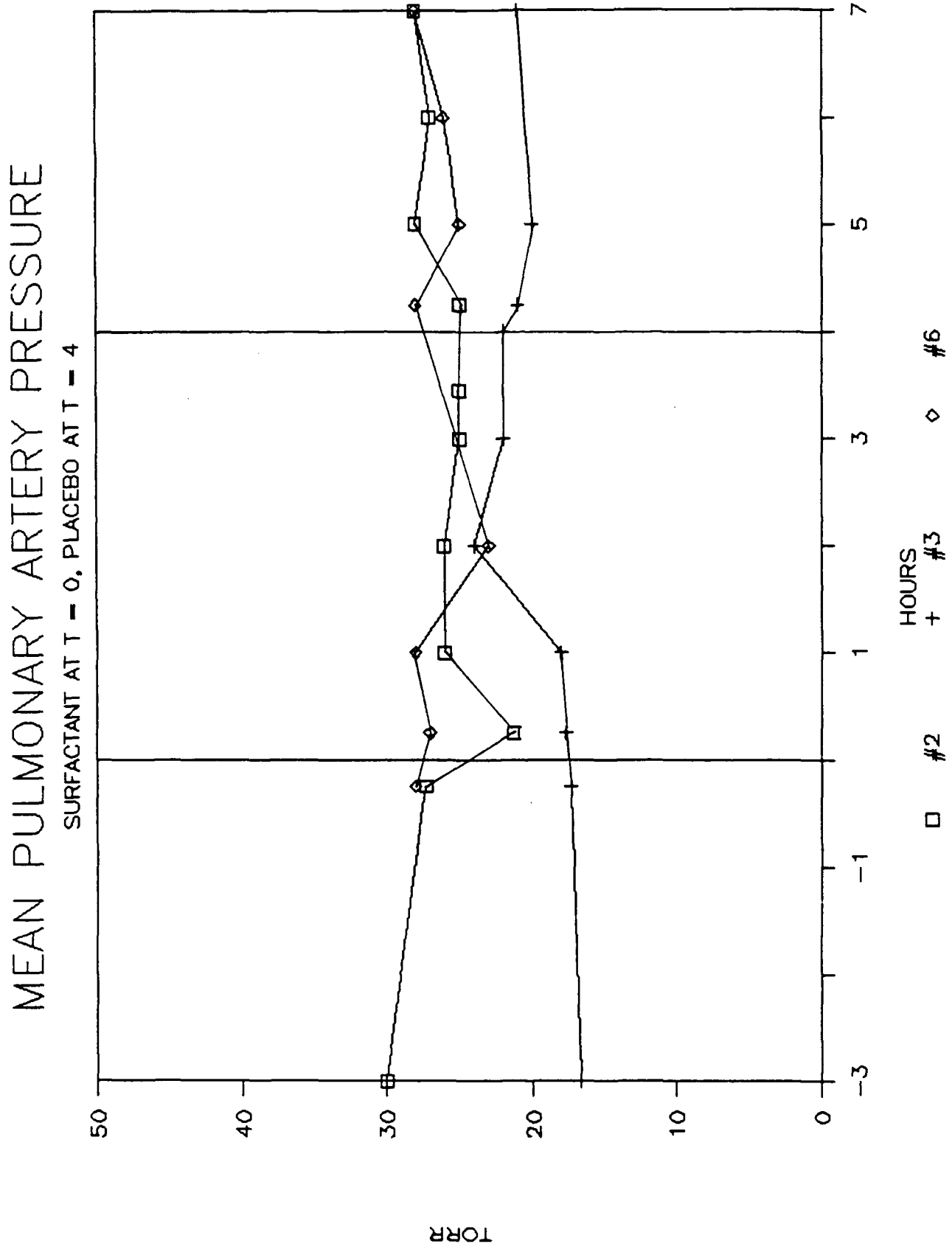

MEAN PULMONARY ARTERY PRESSURE
SURFACTANT AT T = 0, PLACEBO AT T = 4
50
40
30
20
10
0
TORR
-3
-1
1
3
5
7
HOURS
□ #2
+ #3
◇ #6

FIGURE 19

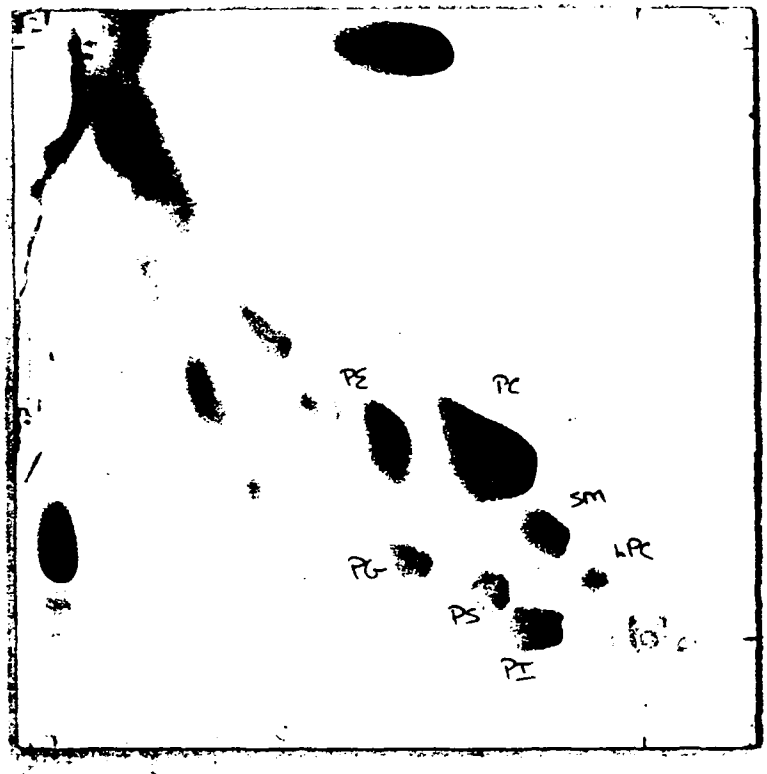
THIN LAYER CHROMATOGRAPH: Patient #5; BAL after Treatment #2

FIGURE 20

THIN LAYER CHROMATOGRAPH: Patient #6 Baseline BAL

FIGURE 21

THIN LAYER CHROMATOGRAPH: Patient #6; BAL after Treatment #1



APPENDIX III: PUBLICATIONS

The clinical pilot projects supported by this contract required completion prior to preparation for publication. Such preparation is now in progress, and will result in at least two additional publications relevant to the content of the contract work. In addition, preliminary reports and efforts supported in part by resources of this contract have been published and are listed below.

1. Smith, R.M. and Spragg, R.G. Production and Administration to Dogs of Aerosols of Alpha-1-Proteinase Inhibitor. Am J. Med., 84:6A,48-51, 1988.

2. Moser, K.M., Smith, R.M., Spragg, R.G., Tisi, G.M. Intravenous Administration of Alpha-1-Proteinase Inhibitor in Patients with PiZ and PiM Phenotype: Preliminary Report. Am. J. Med., 84:6A, 70-74, 1988.

3. Merritt, T.A., Strayer, D.S., Hallman, M., Spragg, R.G., Wozrniak, P. Immunologic Consequences of Exogenous Surfactant Administration. Semin. Perinatol., 12:221-230, 1988.

4. Spragg, R.G., Richman, P., Gilliard, N., Merritt, T.A. The Future of Surfactant Therapy of the Adult Respiratory Distress Syndrome in Surfactant Replacement Therapy in Neonatal and Adult Respiratory Distress Syndrome B. Lachmann, ed., Springe Verlag Berlin Heidelberg, Germany, p 203-211, 1988.

5. Strayer, D.S., Merritt, T.A., Spragg, R.G., Hallman, M.: Immunogenicity of Surfactant and Its Implications for Replacement Therapy. in Surfactant Replacement Therapy in Neonatal and Adult Respiratory Distress Syndrome,B. Lachmann, ed., Springe Verlag Berlin Heidelberg, Germany, p 301-313, 1988.

6. Auger, W.R., Smith, R.M., Spragg, R.G. Protease Release and Anti-Protease Inactivation in a Clinical Model of Acute High Permeability Lung Injury. Am. Rev. Respir. Dis., 137:A146, 1988.

7. Richman, P.S., Spragg, R.G., Robertson, B., Merritt, T.A., Curstedt, T. The Adult Respiratory Distress Syndrome: First Trials with Surfactant Replacement. Eur. J. Respir. Dis., 12:221-30, 1989.

8. Smith, R.M., Traber, D.L., Traber, L., Spragg, R.G. Pulmonary Deposition and Clearance of Aerosolized Alpha-1- Proteinase Inhibitor Administered to Dogs and Sheep. J. Clin. Invest., 84: 1145-1154, 1989.

9. Spragg, R.G., Richman, P., Gilliard, N., Merritt, T.A. The Use of Exogenous Surfactant to Treat Patients with Acute High-Permeability Lung Edema. Prog. Clin. Biol. Res., 308:791-796, 1989.

10. Merritt, T.A., Hallman, M., Spragg, R.G., Heldt, G.P., Gilliard, N. Exogenous Surfactant Treatments for Neonatal Respiratory Distress Syndrome and their Potential Role in the Adult Respiratory Distress Syndrome. Drugs 38:591-611, 1989.

11. Merritt, T.A., Strayer, D.S., Hallman, M., Spragg, R.G.: Immunologic Considerations Regarding Exogenous Surfactants. in Surfactant Replacement Therapy D.L. Shapiro, R.H. Notter, eds, Alan R. Liss, Inc, New York, New York, p 145-162, 1989.

12. Gilliard, N., Spragg R.G. Pulmonary Distribution of Exogenous Surfactant in Rabitts - Effect of Instilled Volume. Am. Rev. Respir. Dis., 139:A274, 1989.

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